ENVIRONMENTAL FACTORS INFLUENCING GROWTH, FREEZING TOLERANCE AND PHOTOSYNTHESIS IN *Thellungiella* AND *Arabidopsis*

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ABSTRACT

Thellungiella salsuginea is an *Arabidopsis* related, promising model plant for stress tolerance studies. This study aimed to characterize environmental responses of Yukon and Shandong ecotypes of *T. salsuginea* in comparison with *Arabidopsis thaliana* – Columbia ecotype. The study comprised an analysis of plant growth, freezing tolerance, photosynthesis and respiration under various growth regimes. The experimental tools and techniques included visual observation, physical measurements, electrical conductivity, spectrophotometry, chlorophyll *a* fluorometry, P_{700} spectroscopy and oxygen-electrode polarography. The taxa showed several commonality and divergence in physiological responses. From the similarity of constitutive freezing tolerance (lethal temperature for 50% plants, LT_{50} = -7.5 ˚C) between the taxa, cold acclimation induced highest level of cold tolerance in Yukon (LT_{50} =-21˚C) followed by Shandong (LT_{50} = -17˚C) and *Arabidopsis* (LT_{50} = -14˚C). A drought for four days followed by cold acclimation for one week furthered LT_{50} to -25^{o}C in Yukon and Shandong, but not in the *Arabidopsis*. Yukon and Shandong required either vernalization or ecotype-specific irradiances for the reproductive transition, while *Arabidopsis* underwent flowering across varied growth regimes. The *Thellungiella* ecotypes also contrasted from *Arabidopsis* in photosynthetic properties with their higher chlorophyll and carotenoid contents, higher constitutive resistance to photoinhibition and higher amount of oxidizable photosystem I reaction-center chlorophyll (P_{700}^{+}) than that of *Arabidopsis*. Yukon distinguished itself from other taxa with its higher growth sensitivity to daily photon irradiance, greater acclimative gain in freezing tolerance, higher rates of dark respiration and lower net assimilation, while Shandong exhibited higher electron transport through photosystem II, greater fraction of excitation energy partitioning towards photosystem II photochemistry and contrasting trend of P_{700}^{+} due to cold acclimation. In Shandong, the P_{700}^{+} seemed to decrease due to cold acclimation at higher irradiances, while it appeared to increase in Yukon and *Arabidopsis*. The findings revealed that Yukon and Shandong possessed wider phenotypic plasticity than that of *Arabidopsis*. Contrasting manifestation of environmental responses including the inducible freezing tolerance, reproductive transitioning and carbon economy promise *Thellungiella* to be the subject of great scientific curiosity to unravel the extremophilic adaptation of plants. On the other hand, the physiological versatility of *Arabidopsis* in the moderate environment substantiated its preferential use as a model system representing mesophytic adaptation.

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DEDICATION

This thesis is dedicated to my mother who departed when I was 7, uttering her last words about continuation of my study. It is also dedicated to my late father, who shouldered sole parenting responsibility, becoming my role model for integrity, diligence and philanthropy.

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LIST OF ABBREVIATIONS

λ_{max}	The wavelength at which the maximum fraction of light is absorbed
Φ_{NO}	Efficiency of constitutive non-photochemical energy dissipation and fluorescence
Φ_{NPQ}	Efficiency of light dependent NPQ
Φ_{PSII}	PSII operating efficiency
ΔA_{820}	Light induced change in absorbance at 820 nm
$1\text{-}q_L$	Excitation pressure
ANOVA	Anaysis of variance
AOX	Alternative oxidase
ATP	Adenosine triphosphate
C_3 plants	The plants in which the CO_2 is first fixed into a compound containing three carbon atoms before entering the Calvin cycle of photosynthesis
C_4 plant	A plant in which the CO_2 is first fixed into a compound containing four carbon atoms before entering the Calvin cycle of photosynthesis
CACM	Cold acclimated cold-measured
CarLA	Carotenoid content per unit leaf area
CarW	Carotenoid content per unit leaf weight
CAWM	Cold acclimated warm-measured
CBF	C-repeat binding factor
$_C$DNA	Complementary deoxy-ribonucleic acid
Chl *a:b*	Chlorophyll *a* to chlorophyll *b* ratio
Chl:Car	Chlorophyll to carotenoid ratio
ChlLA	Chlorophyll content per unit leaf area
ChlW	Chlorophyll content per unit leaf fresh weight
CO_2	Carbon dioxide
CP	Chlorophyll binding proteins
Cyt	Cytochrome
D1	One of the proteins of PSII core heterodimer
DAS	Days after sowing
DAT	Daily average temperature

DMRT	Duncan's multiple range test
DPI	Daily photon irradiance
DW	Shoot dry weight
e^-/P_{700}	Intersystem electron transport
ESTs	Expresed sequence tags
ETR_{PSII}	Non-cyclic electron transport rate through PSII
F'	Fluorescence emission from light-adapted leaf
Fd	Ferredoxin
F_m	Maximal fluorescence in the dark-adapted state
F_m'	Maximal fluorescence in the light-adapted state
F_m'/F_m	Ratio of the non-photochemical rate constants in the dark- and light-adapted states
F_o	Minimal fluorescence in the dark-adapted state
F_o'	Minimal fluorescence in the light-adapted state
F_q'/F_m'	PSII operating efficiency
F_q'/F_v'	PSII efficiency factor
FR	Far-red light
F_v/F_m	Maximum quantum efficiency of PSII photochemistry
F_v	Variable fluorescence in the dark-adapted state ($F_m - F_o$)
F_v'	Variable fluorescence in the light-adapted state ($F_m'-F_o'$)
F_v/F_m	Maximum quantum efficiency of PSII photochemistry [$(F_m - F_o)/F_m$]
FW	Shoot fresh weight
HSP	Heat shock protein
Jmax	Activation energy of the rate of RuBP regeneration
KCN	Potassium cyanide
LA	Leaf area
LAR	Leaf area ratio
LCP	Light compensation points
LDMC	Leaf dry matter content
LED	Light emitting diode
LHC	Light harvesting complex
LMA	Mass per unit area

LMF	Leaf mass fraction
LSD	Least significant difference
LT	Leaf thickness
LT_{50}	Lethal temperature (killing 50% of a population)
MGDD	Modified growing degree days
MT	Multiple turnover flash
n	Number of samples; Haploid chromosome number
NACM	Non-acclimated cold-measured
NAD^+	Nicotinamide adenine dinucleotide – oxidized form
NADH	Nicotinamide adenine dinucleotide – reduced form
NADH DH	NADH dehydrogenase complex
$NADP^+$	Nicotinamide adenine dinucleotide phosphate – oxidized form
NADPH	Nicotinamide adenine dinucleotide phosphate – reduced form
NAWM	Non-acclimated warm-measured
ND	NAD(P)H dehydrogenase
NIR	Near-infrared
NPQ	Nonphotochemical quenching ((F_m/F_m') – 1)
NRL	Number of rosette leaves
O_2	Oxygen
P	A measure of consistency between the results actually obtained in the trial and the pure chance explanation for those results
P_{700}	PSI reaction-center chlorophyll *a* molecule with peak absorption spectrum at 700 nm
P_{700}^+	Oxidized P_{700}
PAR	Photosynthetically active radiation
pH	Hydrogen ion concentration
P_{max}	Light-saturated rates of photosynthesis
PMSR	Peptide methionine sulfoxide reductase
PPFD	Photosynthetic photon flux density
PQ	Plastoquinone
PQ	Plastoquinone

PQH_2	Plastoquinol
PsbS	Photosystem II subunit S
PSI	Photosystem I
PSII	Photosystem II
PTOX	Plastid terminal oxidase
Q_{10}	Factor by which respiration increases for a 10°C change in temperature
Q_A	Primary quinone electron acceptor of PSII
q_E	Energy dependant quenching
q_N	Non-photochemical quenching coefficient
q_O	Basal fluorescence quenching coefficient
q_T	State-transition quenching
r	Pearson correlation coefficient
RGR	Relative growth rate
ROS	Reactive oxygen species
RR	Rosette radius
Rubisco	Ribulose-1,5-bisphosphate caboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
RWC	Relative water content
SE	Standard error
SHAM	Salicylhydroxamic acid
SIMR	Stress-induced morphogenic response
SLA	Specific leaf area
ST	Single-turnover flash
STN	Serine-Threonine kinase
TCA	Tricarboxylic acid
UCP	Uncoupling proteins
ULR	Unit leaf rate
UV-B	Ultra-voilet B
$V_c max$	Activation energy of the maximum rate of RuBP carboxylation
α-TQ	α-tocopherol quinone

1.0 INTRODUCTION

Arabidopsis thaliana (thale cress or mouse-ear cress) has been widely recognized as the model plant in experimental biology (Koornneef and Meinke 2010). It belongs to Brasicaceae family consisting of about 310 genera and 3500 species with diverse growth forms, seasonality and geographical distribution in the world (Al-Shehbaz 2011). Preferential use of *A. thaliana* (hereinafter referred to as *Arabidopsis*) as the model system of experimental biology is attributed to its wide geographical distribution with vast genetic diversity, short life cycle, self-pollination, high fecundity, small genome size, cultivability in diverse media, genetic transformability and mutagenesis that has led to the rapid generation of a wealth of information along with the complete genetic sequence of the species (Al-Shehbaz and O'Kane 2002; Pigliucci 2002; Berardini and Rhee 2004; Bevan and Walsh 2005). However, being a glycophytic plant, *Arabidopsis* has a relatively low capacity to survive in extreme abiotic stresses (M'rah et al. 2006; Ghars et al. 2008) and that limits its use for stress-physiological studies. Therefore, searches for alternative or complimentary model systems are always underway with the hope of gaining a greater understanding of abiotic stress tolerance mechanisms in plants (Inan et al. 2004; Amtmann et al. 2005; Wong et al. 2005; Amtmann 2009).

Thellungiella salsuginea (salt-water cress) (hereinafter referred to as *Thellungiella*) has been identified as an *Arabidopsis*-related, extremophilic species with promising attributes as a stress-tolerant plant model system. Two ecotypes of *Thellungiella*, namely Yukon and Shandong, have received growing scientific interest for their high genetic and phenotypic similarities with *Arabidopsis* and at the same time their higher tolerance to salinity (Bressan et al. 2001; Inan et al. 2004; Wong et al. 2005, 2006), cold (Griffith et al. 2007) and nutritional deficiency (Kant et al. 2008) than that of *Arabidopsis*. These ecotypes were evolved in contrasting stress-prone ecophysiological habitats. The *Thellungiella* Yukon ecotype (hereinafter referred to as Yukon) was collected from sub-arctic, semi-arid habitat of alkaline, saline meadows underlain by permafrost at the Takhini river valley, 40 km west of Whitehorse (60°51.29'N 135°43.04'W) in the Yukon Territory of Canada. Presumably, the adaptation of this ecotype in the sub-arctic, short growing season with simultaneous, multiple stresses has required the evolution of dynamic mechanisms to tailor its growth and phenology against extreme climatic and edaphic constraints (Wong et al. 2005; 2006; Griffith et al. 2007; Kant et al. 2008; Amtmann 2009). The *Thellungiella* Shandong ecotype (hereinafter referred to as Shandong) naturally grows in the high-salinity coastal areas of Shandong Province in north-eastern China. This ecotype is known to have extreme degree of salinity tolerance and

has been used as a model halophyte in many studies (Taji et al. 2004; Amtmann et al. 2005; M'rah et al. 2006; Amtmann 2009; Wang et al. 2010).

The contribution of *Arabidopsis* in enhancing the efficiency of plant biological research can hardly be over-emphasized. However, glycophytic nature of *Arabidopsis* constrains both scientific curiosity and practical relevance of the studies that go beyond the adaptive limits of *Arabidopsis*. Identification of extremophillic plant model systems from close relatives of *Arabidopsis* can, therefore, overcome the experimental constraints associated with *Arabidopsis*, while capitalizing on the existing knowledge and resources generated from *Arabidopsis*-led initiatives (Inan et al. 2004; Amtmann et al. 2005; Wong et al. 2005; 2006; Volkov and Amtmann 2006; Griffith et al. 2007; Ghars et al. 2008; Kant et al. 2008; Amtmann 2009; Oh et al. 2009; 2010; Arbona et al., 2010; Orsini et al. 2010; Pang et al. 2010; Wang et al. 2010). As diverse relatives of *Arabidopsis* include some economically important crops (Al-Shehbaz 2011), use of its extremophillic relatives for stress-tolerance studies enhances the practical relevance of these studies to crop adaptation (Prato et al. 2010), phytoremediation (Arthur et al. 2005) and biodiversity conservation (Linke and Norris 2003).

While there is growing surge of studies using *Thellungiella*, primarily the Shandong ecotype, a reference database of their growth, phenology and environmental responses is still lacking. There are outstanding questions regarding to what extent stress (freezing) tolerance, growth, phenology, and the prime determinants of the carbon economy of plants, photosynthesis and respiration, are comparable between *Arabidopsis* and its experimental alternatives Yukon and Shandong. This question underlies the scientific curiosity as to whether physiological potential of *Thellungiella* promises to offer comparative advantages to *Arabidopsis* to extend the understanding of this plant under different experimental conditions. In this study, these scientific curiosities were asserted in the form of the hypothesis as: If Yukon, Shandong and *Arabidopsis* are adapted to contrasting ecological conditions selecting for distinctive physiological competence, then these taxa will display distinctive responses to temperature irradiance and photoperiod for the growth and development, photosynthesis, respiration and freezing tolerance parameters.

1.1 Thesis Objectives

The general objective of the study was to contribute to the enhancement of physiological information base for the use of *Thellungiella* as an extremophillic model plant for the elucidation of abiotic stress tolerance in plants. The specific objectives, guided by the above hypothesis were:

- to demonstrate comparative freezing tolerance and acclimation capacity of *Thellungiella* ecotypes and *Arabidopsis* (Columbia ecotype);
- to characterize the growth behaviour of *Thellungiella* ecotypes in comparison with *Arabidopsis* (Columbia ecotype) under different growth regimes;
- to elucidate comparative photosynthetic and respiratory properties of *Thellungiella* ecotypes and *Arabidopsis* (Columbia ecotype) under different growth regimes.

In pursuit of the above objectives, the study covered the aspects of freezing tolerance, growth, photosynthesis, and respiration of Yukon, Shandong and *Arabidopsis* under different growth regimes varying in temperature, irradiance and photoperiod. The Columbia ecotype (Col-0) of *Arabidopsis* was used in this comparative study, as this ecotype is considered as the reference model in the experimental biology (Koornneef and Meinke, 2010). The Col-0 ecotype is hereinafter referred to as *Arabidopsis*. The growth regimes used in this study were reference mesophytic and cold-acclimating conditions frequently adopted in previous studies and lied within the adaptive range of *Arabidopsis.*

The study results are presented in the four thematic chapters covering freezing tolerance, growth, photosynthesis and respiration. The first thematic chapter contains a detailed comparative characterization of freezing tolerance in Yukon, Shandong and *Arabidopsis* along with relative efficacy of chlorophyll fluorescence imaging as an alternative method for the determination of freezing tolerance and screening populations for this characteristic. The next chapter on growth examines phenotypic plasticity within and across different growth regimes presenting comparative developmental phenology, and relative and absolute growth parameters. Similarly, the chapter on photosynthesis presents the comparative results of the photosynthetic plasticity demonstrated by photosystem I (PSI) and photosystem II (PSII) in response to short- and long-term changes in experimental conditions. Finally the fourth thematic chapter gives a comparative account of dark respiration of the three experimental taxa. These chapters are preceded by a Literature Review covering the broad ecophysiological background of the experimental taxa and general aspects of plant growth, phenotypic plasticity, acclimation, adaptation and finally photosynthetic and respiratory adjustments and interactions. The findings of all four data chapters are synthesized in a General Discussion with particular reference to the relative advantage of Yukon, Shandong and *Arabidopsis* for their use as model systems for different physiological specificities. Finally, conclusions are generated from the findings of the study with the recommendations for further research.

2.0 LITERATURE REVIEW

2.1 The Model Plants: *Thellungiella* and *Arabidopsis*

2.1.1 Taxonomy

Thellungiella and *Arabidopsis* are the closely related genera of Brassicaceae family. The Brassicaceae family is subdivided into 44 tribes, 310 genera and 3,500 species (Al-Shehbaz et al. 2006; Bailey et al. 2006; Warwick et al. 2010; Al-Shehbaz 2011). Some of the species of this family are economically important cultivated plants, while some others such as *Arabidopsis* and *Thellungiella* are the model plants of experimental biology (Al-Shehbaz 2011 ; Amtmann et al. 2005; Amtmann 2009; Koornneef and Meinke 2010).

The phylogeny of the members of the family Brassicaceae is yet to be precisely resolved. With the use of molecular phylogenetic techniques, the traditional morphogenic classification has been changed over time and the species within the Brassicaceae family have undergone regrouping under different taxa (Al-Shehbaz et al. 2006; Bailey et al. 2006; Warwick et al. 2010). The name *Arabidopsis* was originally proposed in 1821 by de Condolle (cited in Al-Shehbaz and O'Kane 2002) and its taxonomic identity was resolved relatively recently after its biological importance was established (Al-Shehbaz and O'Kane 2002). *Arabidopsis* and *Thellungiella* are phylogentically close taxa with similar morphology, life history and reproductive properties. The distinguishing morphogenic traits of *Arabidopsis* include short petiolated leaves that are either sagittate or auriculate at the base, simple forked trichomes, fruit valves with a prominent midvein and usually wingless seeds. Similarly, the characteristic morphogenic features of *Thellungiella* include completely glabrous plants with glaucous leaves and stems and adaptation to high salinity (Al-Shehbaza and O'Kane 2002**).**

Currently the genus *Arabidopsis* consists of 11 species (Al-Shehbaz et al. 2006), of which *Arabidopsis* has been most extensively characterized model plant (Koornneef and Meinke 2010). Similarly, the genus *Thellungiella* has been classified into four species including *T. salsuginea* (Pallas) O.E. Schulz, *T. halophila* (C.A. Meyer) O.E. Schultz, *T. parvula* (Schrenk) Al-Shehbaz and O'Kane and *T. Botschantzevii* (Amtmann 2009). However, some authors consider *T. salsuginea* to be synomymous to *T. halophila* (Amtmann et al. 2005; Wong et al. 2005; Griffith et al. 2007) to which two designated extremophillic model ecotypes, Shandong and Yukon, belong.

2.1.2 Ecophysiology

Both *Arabidopsis* and *Thellungiella* are herbaceous annuals distributed in the temperate and alpine climates in the northern hemisphere from East Asia through Europe to North America. Although *Arabidopsis* is a glycophytic plant growing under moderate environmental conditions, it has much wider geographical span of adaptation ranging from the high mountains of equatorial Africa and Himalayan highlands of South Asia to northern Europe up to 68°N (Li et al. 1998; Hoffmann 2002; Zhen and Ungerer 2008). Biogeographic analysis done by Hoffmann (2002) showed that the climatic factors limiting its distribution include low temperatures during spring and autumn and high temperatures exceeding monthly average of 22°C, coupled with low precipitation in summer. In contrast, *Thellungiella* are adapted to the more extreme ecological niches, usually presenting multiple abiotic stresses such as high salinity, cold, soil nutrient imbalances and erratic moisture regimes (Amtamann 2009; Oh et al. 2010; Orsini et al. 2010).

Annual plant species are classified into summer annuals and winter annuals. Summer annuals have shorter lifecycle that starts with germination in the warm spring and completes with the maturity in a few short months in the summer. On the other hand, in winter annuals seed germination takes place in the fall and the seedlings overwinter leading to onset of flowering in the spring. *Arabidopsis* is predominantly a winter-annual species, but there are *Arabidopsis* ecotypes with summer-annual growth patterns (Grennan 2006).

Arabidopsis accessions display tremendous variations in their ecophysiological traits along the latitudinal gradient of their origin. In general, the accessions from higher latitudes tend to flower late and are typically winter annuals, while the accessions from lower latitudes possess both summer and winter annual features (Johanson et al. 2000; Stinchcombe et al. 2004; Stinchcombe et al. 2005). The latitude of origin had significant negative correlation with plant size and relative growth rate (Li et al. 1998), and a significant positive correlation with freezing tolerance, cold acclimation capacity (Zhen and Ungerer 2008) and days to bolting (Stinchcombe et al. 2004). *Arabidopsis* ecotypes display differential sensitivity to vernalization, the process in which flowering is triggered by a prolonged exposure to low temperature. Several loci controlling vernalization-mediated flowering are known, of which the *FRIGIDA* locus and its interaction with *FLOWERING LOCUS C* is commonly responsible for the vernalization sensitivity in this species (Johanson et al. 2000; Stinchcombe et al. 2004, 2005). The association between the geographical origin and ecophyiological responses of the accessions suggests the role of natural selection in creating adaptive variation.

The Columbia ecotype of *Arabidopsis* is considered as the reference model for the availability of wealth of information and resources including physiological and biochemical knowledge, high quality genetic sequence and micro-array data, and extensive collections of mutants (Koornneef and Meinke, 2010). The geographical origin of this ecotype is not very clear (Johanson et al. 2000). The original Columbia ecotype was selected by Redei in 1992 (cited in Koornneef and Meinke 2010) from the non-irradiated Landsberg accession collected in the Landsberg an der Warthe (Gorzów Wielkopolski) region of Poland (Koornneef and Meinke, 2010). Since then, this ecotype has gone through numerous cycles of artificial selections in the laboratory studies (Wilczek et al. 2010).

Recently, two ecotypes of *Thellungiella,* namely Yukon and Shandong, have emerged in abiotic stress studies. The Yukon ecotype was collected from the sub-arctic, semi-arid habitat of alkaline, saline meadows underlain by permafrost, at the Takhini river valley, 40 km west of Whitehorse (60°51.29'N 135°43.04'W) in Yukon Territory of Canada. Having evolved in this habitat of simultaneous, multiple stresses with a short growing season, the Yukon ecotype is believed to have a constitutive mechanism to tolerate extreme cold, salinity, drought, mineral toxicity and/or nutrient deficiency (Wong et al. 2005; 2006; Griffith et al. 2007; Kant et al. 2008). In its natural habitat, the Yukon ecotype grows as the summer-annual with annual variation in plant phenotypes from dwarf to a well-grown tall stature depending on the weather conditions of the particular year (Amtmann 2009).

Another extremophillic ecotype of *Thellungiella*, known as the Shandong ecotype, is a winter-annual plant growing naturally in the high-salinity coastal areas of Shandong Province in north-eastern China. This ecotype possesses a winter-annual growth habit with an obligatory vernalization requirement. Six weeks of low temperature (4°C) treatment is effective in triggering flowering and it takes about 10 months to flower without vernalization (Inan et al. 2004; Fang et al. 2006). This ecotype is known to have extreme degree of salinity tolerance and used as a model halophyte (Inan et al. 2004; Taji et al. 2004; Amtmann et al. 2005; M"rah et al. 2006., Amtmann 2009; Wang et al. 2010; Oh et al. 2010; Orsini et al. 2010).

2.1.3 Genetics and Molecular Biology

The genus *Arabidopsis* is genetically diverse with different genome size and ploidy levels. The diploid species in the *Arabidopsis* genus have 5 or 8 pairs of chromosomes (2n = 10 or 16), while some other species are tetraploids with (2n = 26) (reviewed by Koornneef et al. 2004). *Arabidopsis* is a diploid species with 5 pairs of chromosomes (2n = 10) with one of the smallest nuclear genome sizes among the

flowering plants (Bennett and Leitch 2005; Oyama et al. 2008). *Arabidopsis* has an estimated genome size of about 146 Mb with 26,207 predicted protein coding genes. With some 2,330 alternatively spliced genes, there are about 27,855 distinct proteins that are estimated to be expressed in this species (Bevan and Walsh 2005). Some 3,786 transposons and pseudogenes are also estimated that are implicated for the genome size alteration over generations (reviewed by Bevan and Walsh 2005; Oyama et al. 2008). *Arabidopsis thaliana* was the first plant species to have its genome sequence completed (The *Arabidopsis* Genome Initiative 2000). The availability of the *Arabidopsis* genome sequence and resources, including expressed sequence tags (ESTs), full-length complementary deoxyribose nucleic acid (cDNAs) and tagged mutants have revolutionized experimental plant biology (Alexandrov et al. 2006). Furthermore, the 1001 *Arabidopsis* Genomes Project is currently underway towards analyzing the genome sequence variation in 1,001 accessions of *Arabidopsis* (Weigel and Mott 2009).

The genetic properties of *Thellungiella* are quite comparable to those of *Arabidopsis*. *Thellungiella* has seven pairs of chromosomes and the genome size is estimated to be 260 Mb, which is about 1.8 times the size of the *Arabidopsis* genome (Inan et al. 2004; Orsini et al. 2010). Similar to *Arabidopsis*, the presence of varied repetitive sequences including long terminal repeat retroelements, DNA transposons and simple sequence repeats are common in *Thellungiella* (Deng et al. 2009; Nah et al. 2009). The comparison of expressed sequence tags and cDNAs between the two species have revealed over a 90% sequence identity for most genes (Inan et al. 2004; Wang et al. 2004; Wong et al. 2005; Fang et al. 2006; Taji et al. 2008) with a high degree of micro-colinearity between the orthologous regions of the two genomes, despite the fact that both genomes have undergone continuous alterations in the DNA through translocation, loss, or inversion of the nucleotide sequences (Bevan and Walsh 2005; Deng et al. 2009; Nah et al. 2009).

Thellungiella and *Arabidopsis* display comparable transcriptomic and proteomic characteristics in response to abiotic stress (Taji et al. 2004, 2008; Inan et al. 2004; Wang et al. 2004; Wong et al. 2005; Pang et al. 2010). For example, both species undergo the expression of cold responsive genes of c-repeat binding factor (*CBF*) regulon and genes involved in salinity tolerance (*SALT OVERLY SENSITIVE1, SOS1*) upon the exposure to these respective stresses (Griffith et al. 2007; Oh et al. 2010). However, unlike in *Arabidopsis*, many of the universally stress-responsive genes tend to be expressed constitutively in *Thellungiella*. Furthermore, the stress responses in *Arabidopsis* are characterized by more global patterns of transcriptional change (Ma and Bohnert 2007; Nakashima et al. 2009), while *Thellungiella* displays

stress-specific gene expression along with some novel genes that are specific to *Thellungiella* (Taji et al. 2004; 2008; Wang et al. 2004; Gong et al. 2005; Wong et al. 2005; 2006; Kant et al. 2006).

2.2 Plant Growth

2.2.1 Morphogenic Growth Kinetics

Plant growth is defined as the irreversible increase in mass, size or volume of a tissue, organ or a plant (Walter et al. 2009). It is a highly dynamic and regulated outcome of the interplay between the genetic and environmental factors. While the major framework of plant architecture and functional attributes of a plant species are genetically pre-determined (Poorter et al. 1990; Garnier 1992; Reich et al. 1997), plants have the mechanisms to regulate their growth rate, pattern and phenology in response to environmental conditions (Reich et al. 2003; Walter and Schurr 2005; Walter et al. 2009; Wolters and Jürgens 2009). Plant growth is driven by photosynthesis that generates metabolic energy and carbon skeletons for the assimilation of mineral nutrients (Schurr et al. 2006; Smith and Stitt 2007). The environmental conditions dictate the extent to which plant processes function and resources are available for biosynthetic and metabolic processes. Plants, in turn, have the ability to regulate resource demand, adjust the ontogeny, and alter the allocation of bio-assimilates for various competing sinks (Chapin III et al. 1987; Matyssek et al. 2005; McCarthy and Enquist 2007).

Despite the diversity in plant growth architecture and functional strategies, plants display some degree of convergence in morphogenic growth in response to the environment. Limiting light conditions or exposure to shade causes a preferential allocation of biomass to the shoot leading to photomorphogenetic responses, whereas the condition of limiting mineral nutrient(s) favours allocation to the root to optimize resource capture (Shipley and Meziane 2002). The redistribution of plant biomass between the plant organs leads to the alteration of root-to-shoot ratios (Gedroc et al. 1996). In conditions of mild chronic stress, plants exhibit characteristic stress-induced morphogenic response (SIMR) phenotypes as the result of the reorientation of cell division, elongation and differentiation patterns (Pasternak et al. 2005; Potters et al. 2007; 2009; Zolla et al. 2010). Reich et al. (1997) found interspecies similarity of relationships among leaf structures, functions and growth across ecological biomes spanning from tropics to tundra. The rates of photosynthesis and respiration were positively correlated with leaf nitrogen concentration and leaf surface area-to-mass ratio, and negatively correlated with the leaf life-span. Consequently, the productivity of individual plants and of leaves in vegetation canopies also held similar correlations with leaf life-span and surface area-to-mass ratio (Reich et al. 1997).

Plant species display a generally unifying pattern of growth kinetics. The time course of change in biomass of individual plants follows a characteristic sigmoidal fashion with three distinguishable phases of growth. The initial phase is characterized by an exponential increase in plant biomass which is followed by a steady increase in plant biomass as plants grow and finally reaching the asymptotic state of growth towards maturity (Hunt 1990; 2003; Yin et al. 2003). Several sigmoidal growth models with biologically interpretable parameters have been proposed to explain the growth of individual plants (Richards 1959; Birch 1999; Yin et al. 2003). These models contain biologically interpretable parameters with varying degree of relaxation of the logistic curve's restrictions (Tsoularis and Wallace 2002).

2.2.2 Plant Growth Analysis

Individual plant growth involves the quantitative change in various functional traits (Körner 1991; Cornelissen et al. 2003). The changes can be in the form of weights, areas, volumes or contents of plants or plant components. Growth analysis provides a quantitative view of plant performance in a given circumstance (Poorter et al. 1990; Hunt and Cornelissen 1997; Poorter 1999; 2002). Plant growth rate can be expressed in absolute or relative terms. In the absolute measure, the growth rate is expressed as the change in weight or number of plants or plant organs over specified period of time. The relative growth indices are derived through the expression of changes in one variate with respect to the change in other variate in a given time. The relative growth indices are independent of scales of organism and hence allow interspecific comparison of innate growth potentials. Some of the growth indices are also expressed as the simple ratios between the growth variates (Hunt 2003). Major relative growth indices and ratios include relative growth rate (RGR, increase in dry mass per unit of plant mass over a specified period of time), unit leaf rate (ULR, growth rate per unit leaf area), specific leaf area (SLA, leaf area per unit dry mass), leaf mass per area (LMA, leaf dry mass per unit area), leaf mass fraction (LMF, leaf mass per unit of total plant mass), leaf area ratio (LAR, the quotient of total leaf area and total dry weight), and the root-shoot allometric coefficient (a ratio between root and shoot mean relative growth rates) (Hunt 1990; 2003; Poorter 2002).

There are inherent interrelationships between various growth indices (Garnier 1992). RGR is essentially the product of ULR, SLA and LMF (Hunt and Cornelissen 1997; Poorter 2002). In herbaceous plants, RGR is predominantly determined by the ULR and SLA, whereas in woody species, LMF is the major determinant of RGR (Hunt and Cornelissen 1997). RGR and ULR values are higher in annuals than in perennials (Garnier 1992). Plants in productive habitats have inherently higher RGR, while stress and

resource limited conditions result in lower RGR (Poorter 2002). The RGR and its underlying components are correlated with photosynthesis and respiration which are the primary processes determining the carbon economy of the plants (Poorter et al. 1990; Poorter 1999). The growth indices thus reveal physiological performance and ecological strategies of the plants.

There are two approaches to plant growth analysis: the classical approach and the functional approach. In the classical approach the relative growth indices are derived through natural logarithmic transformation of mass and size parameters and then a simple arithmetical solution (Poorter and Garnier 1996; Hunt 2003). Hunt et al. (2002) developed a simple but all-inclusive software tool that generates the values of major relative growth indices with the input of primary data. The functional approach on the other hand, involves curve fitting with the use of non-linear asymptotic functions to derive the instantaneously defined terms (Poorter and Garnier 1996; Hunt 2003). Both approaches have their strengths and limitations and use of combined approach has been suggested (Poorter 1989).

2.3 Plant Acclimation and Adaptation

2.3.1 Phenotypic Plasticity

Structural and functional flexibilities are the requisites in the sessile life of plants. Plants possess inherent genetic potential to tailor their growth, development and morpho-physiological attributes to environmental variation. Plant genotypes exposed to a range of environments display different types and degrees of environmental responses. The range of phenotypes produced by specific genotypes across environmental gradient is called a reaction norm. Phenotypic plasticity is the quantitative change in the reaction norm with respect to the environmental gradient (de Kroon et al. 2005; Valladares et al. 2006; Dechamps et al. 2007; Kramer 2008; Waller et al. 2008). As defined by Pigliucci et al. (2006) phenotypic plasticity is the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions. All higher plants are modular organisms comprising repetitive functional units called modules. Phenotypic plasticity pervades from the module to organismal scale in both quantitative and qualitative traits pertaining to biomass, shape, size, allometry and ontogeny (de Kroon et al. 2005; Valladares et al. 2007). It is essentially the manifestation of the structural and functional flexibilities to fit to the environmental specificity.

Phenotypic plasticity is the output of interactions between genotype and environment. It underlies the cascade of coordinated mechanisms encompassing sensing, processing, transducing and translating environmental signals to result in a reprogramming leading to an array of structural and functional

adjustments in the organism (Kramer 2008; Niklas 2009). Amidst the broad aspects of genetic reprogramming, alternative splicing (Marden 2008), epigenetics (Johnson and Tricker 2010) and cryptic genetic variation (Gibson and Dworkin 2004; Schlichting 2008) are also ascribed for triggering phenotypic plasticity. The amplitude of environment-induced phenotypic variation is controlled by an intrinsic genetic mechanism, called canalization. Canalization buffers the phenotypic variation against mutation and environmental vagaries (Hornstein and Shomron 2006; Hall et al. 2007; Van Buskirk and Steiner 2009). Phenotypic plasticity may be associated with fitness costs or it may underlie evolutionary trade-off (Valladares et al. 2007; Van Buskirk and Steiner 2009; Auld et al. 2010). Canalization is thought to stabilize the architectural and functional robustness of organism in the face of stochastic perturbations and also maintain evolvability by masking the mutational expression (Hornstein and Shomron 2006; Hall et al. 2007).

There is extensive body of literature on the aspect of phenotypic plasticity and the fitness cost of *Arabidopsis* (Pigliucci and Schlichting 1996; Brock and Weinig 2007; Weinig et al. 2006; Sangster et al. 2007; Valladares et al. 2007; Gutierrez et al. 2009). *Arabidopsis* ecotypes possess a phenomenal degree of phenotypic plasticity in various growth and developmental attributes in response to variation in environmental factors including photoperiod, light quality, irradiance, temperature, flooding, nutrient supply, physical contact, competition, herbivory and pathogenesis (Markwell and Osterman 1992; Pigliucci et al. 1995; 2003; Pigliucci and Byrd 1998; Dorn et al. 2000; Bonser and Aarssen 2001; Pollard et al. 2001; Pigliucci 2002; Pigliucci and Kolodynska 2002a; 2002b; Ungerer et al. 2003; Pouteau et al. 2004; Callahan and Pigliucci, 2005; Aguirrezabal et al. 2006; Göllner et al. 2008). *Arabidopsis* has also been used to elucidate the mechanism of canalization and role of heat shock protein (*HSP90*) in the canalization process (Hall et al. 2007; Sangster et al. 2008). Similarly, stress tolerance studies have indicated an appreciable degree of phenotypic plasticity in *Thellungiella* ecotypes (Inan et al. 2004; Griffith et al. 2007; Kant et al. 2008; Amtmann 2009). For example, the Yukon ecotype in its natural habitat shows divergent plant stature in different years; it grows tall and lusty in the years with good precipitation while remaining dwarf and stunted in dry years (Amtmann 2009).

2.3.2 Differences Between Acclimation and Adaptation

Natural environmental factors tend to change continuously in the whole range of temporal and spatial scales. Plants continuously interact with the prevailing environmental factors to maintain physiological homeostasis. The environmental factors set the limit in which plants remain in physiological homeostasis or in which plants need to readjust their physiology to attain a new state of homeostasis. If the

environmental factors exert stress that exceeds the tolerable limits, plants fail to survive (Gaspar et al. 2002; Schulze et al. 2005). Plants possess myriad of functional flexibilities responding to the transient changes in the environmental variability. Plants also have mechanisms to sense environmental cues, signaling relatively longer-term changes in environment and reconfigure their metabolic machinery and developmental strategies through extensive genetic reprogramming, the process known as acclimation (Gray and Heath 2005; Bräutigam et al. 2009; Ahuja et al. 2010; Hummel et al. 2010; Stitt et al. 2010). Exposures to the particular set of environmental conditions over generations leads to the evolution of physiological attributes that enhance fitness of plants in the given ecological conditions, the phenomenon referred to as adaptation (Schulze et al. 2005; Ehrenreich and Purugganan 2006; Kosakivska 2008; Siol et al. 2010).

Acclimation is essentially the expression of phenotypic plasticity of an individual organism during its growth and development, while adaptation is the development of a naturally selected phenotype resulting from changes in the genetic architecture over the generations. The term 'acclimation' thus refers to "the habituation of an individual organism to a different or changing environment" whereas 'adaptation' denotes "the evolutionary process by which a species becomes limited to its environment" (Hovenden 2007). Notwithstanding the fundamental differences in the biological meaning of these two terms, they are found to be used synonymously (and incorrectly) in some literature (Backhausen and Scheibe 1999; Vance et al. 2003; Assunção et al. 2010).

The process of adaptation underlies the natural selection that acts on the genetic variation. Differential genetic recombinations create variation in morphology, anatomy and physiology with differential survival and reproductive success of individuals over successive generations. The genetic recombinants with enhanced fitness of traits to the specific environment are selected for and those with less fitness are selected against (Hovenden 2007; Kimbrell 2010). The allelic frequency governing the plant fitness change in the adaptive process in response to the selection pressure exerted by various abiotic and biotic factors (Ehrenreich and Purugganan 2006; Siol et al. 2010). Thus, the adaptation to the environment is manifested through a permanent change in range of values of a particular character within a population (Geber and Griffen 2003; Banta et al. 2007; Hovenden 2007; Pavey et al. 2010). Plants adapt to their ecological niches with different strategies such as competitors, ruderals or stress-tolerant (Kosakivska 2008). *Arabidopsis* is considered ruderal species (Banta et al. 2007), while *Thellungiella* ecotypes are typified by their stress-tolerant strategies (Amtmann 2009).

The process of acclimation involves readjustment of morphology, anatomy and physiology of an individual organism in response to altered environmental conditions. Unlike the permanent shift in the values of adaptive traits across generations, the acclimatory effects are mostly intra-generational and induced by the exposure to a particular stress factor. Acclimation is said to be dynamic if a plant organ developed in one environment maintains the ability to adjust its physiology in the altered environmental condition. Similarly, acclimation is considered developmental if plants exposed to different environments develop different phenotypic attributes (Athanasiou et al. 2010). Furthermore, the stress induced acclimatory effects may fade away after the subsidence of the inducing factors to the normal degree, a phenomenon known as de-acclimation (Gorsuch et al. 2010). However, there is now growing evidence of transgenerational transmission of some of the stressed-induced acclimatory effects in the form of epigenetic signatures (Molinier et al. 2006; Blodner et al. 2007; Boyko et al. 2010; Richards et al. 2010) and those effects pass to the progeny predominantly through the female rather than male gamete (Boyko and Kovalchuk 2010).

2.3.3 Cold Acclimation

2.3.3.1 Cold Acclimation Capacity

Low temperature is one of the major stress factors affecting the productivity and geographical distribution of plants (Margesin et al. 2007). Low temperature affects the physical and chemical properties of biomolecules leading to the distortion of cellular architecture and metabolic homeostasis in plants. Low temperature stress can be differentiated into two forms: chilling stress caused by low, positive temperatures, and freezing stress caused by sub-zero, negative temperatures. Chilling stress may have a multitude of detrimental effects including metabolic redox disparity, generation of reactive oxygen species (ROS), conformational changes in protein, destabilization of protein complexes, lowering of enzyme kinetics, photosynthetic impairment, membrane rigidification and leakage, cytoskeletal depolymerization and impairment of genetic translation due to the formation of RNA secondary structure (Beck et al. 2007; Ruelland et al. 2009). Freezing stress has additional damaging effects via the alteration of water potential due to freezing. Freezing usually starts with ice nucleation in the apoplast leading to the extracellular ice formation that creates a difference in water potentials between the cell and apoplast. This causes efflux of water to apoplast resulting in cellular dehydration, osmotic contraction of cytoplasm, membrane disruption and finally a loss of plant vitality. A more acute loss of plant vitality may result if freezing occurs inside the cell (Beck et al. 2007; Ruelland et al. 2009).

Plants counter low temperature-induced physiological disturbances through a complex array of survival responses, brought about by cold acclimation. This process involves a suite of activities starting from perception of stress stimuli and signal transduction leading to sometime extensive metabolic reprogramming and reconfiguration of physiology (Arnholdt-Schmitt 2004; Kaplan et al. 2004; Gray and Heath 2005; Wong et al. 2006; Hua 2009; Baena-González 2010; Hirayama and Shinozaki 2010; Janská et al. 2010). After the removal of the stress stimuli, the physiology of the plants tends to revert to normalcy through the process known as de-acclimation. Comparative analysis of gene expression profiles during cold acclimation and de-acclimation of *Arabidopsis* revealed mutually opposite patterns of up-regulation and down-regulation of genes in that 445 genes were up-regulated and 341 genes down-regulated during cold acclimation, while the process of de-acclimation involved up-regulation of 292 genes coupled with down-regulation of 320 genes (Oono et al. 2006). The physiological and genetic changes that have been found to occur during cold acclimation include the production of ribonucleic acid (RNA) chaperones, synthesis of cold responsive and anti-freeze proteins, enzymes and chaperones, induction of ROS scavenging system, photosynthetic non-photochemical quenching, synthesis of photoprotective carotenoids and anthocyanins, increase in photosynthetic enzymes and flux capacity, lipid de-saturation, accumulation of compatible solutes, induction of cold stable microtubules, and increase of cell wall strength and a decrease in the cell wall pore size (Beck et al. 2007; Ruelland et al. 2009; Janská et al. 2010). The ultimate result of these intricate responses is tolerance to low and/or freezing temperatures. The extent of increase in freezing tolerance due to prior exposure to sub-lethal cold temperature is termed as cold acclimation capacity (Ehlert and Hincha 2008; Le et al. 2008; Zhen and Ungerer 2008). Plant species differ in their degree of freezing tolerance and cold acclimation capacity.

2.3.3.2 Freezing Tolerance Assays

Assessment of freezing tolerance is very crucial step in the selection of parents and evaluation of progenies in the crop improvement programs. Identification of plants with exceptional freezing tolerance helps to provide insight about freezing tolerance mechanisms and facilitates the transfer of these traits to target crop varieties. Freezing tolerance of plants can be assessed by using one or more indicators of the physiological effects caused by freezing stress on plants (Calkins and Swanson 1990; Ehlert and Hincha 2008). The regrowth assay and electrical conductivity test are the most widely used methods in freezing tolerance studies. The regrowth method is a more direct and complete approach in which regeneration of plants after the release of freezing stress is taken as the indicator of freezing tolerance. Electrical

conductivity on the other hand, tests the level of leakage of cellular contents from the plant tissues as an indication of the damage to the plasma membrane (Burr et al. 1990; Calkins and Swanson 1990; Warren et al. 1996; Verslues et al. 2006). The major drawbacks associated with these conventional methods are the drudgery and time involved in the processes (Ehlert and Hincha 2008; Woo et al. 2008). Recently, chlorophyll fluorescence imaging, which detects and quantifies spatial variation in the maximum quantum efficiency of PSII photochemistry (F_v/F_m) of the frozen leaves, has been shown to be promising and efficient technique for the evaluation of freezing (Ehlert and Hincha 2008) and drought (Woo et al. 2008) tolerance in plants. The F_v/F_m is at the highest level when there is balance between the generation and utilization of energy, a state known as photostasis. Freezing stress, or even low temperature, destabilizes the photostatic balance leading to the photoinhibition which is reflected by the reduced photochemical efficiency of the plants (Huner et al. 1993; 1998; Gray et al. 2003; Ensminger et al. 2006).

2.3.4 Photosynthetic acclimation

2.3.4.1 Photoinhibition and Photoprotection

Photosynthesis transforms light energy into chemical energy that fuels the autotrophic growth of plants. This involves a coupling of light driven redox chemistry and temperature-dependent enzymatic reactions. Photosystem I (PSI) and photosystem II (PSII) consisting of light harvesting complexes (LHC) and reaction centres are the principle light-processing machineries that work in series with several enzymatic complexes forming an electron transport stream. The chlorophyll and carotenoid molecules associated with the LHC transfer the excitation energy to the reaction centres leading to the oxidation of water into electrons, hydrogen ions (protons) and oxygen. The electrons thus produced flow through the electron transport chain and finally reduce nicotinamide adenine dinucleotide phosphate ($NADP^+$) into NADPH. Similarly the hydrogen ion concentration (pH) gradient built between the lumen and stroma of the chloroplast serves as the proton-motive force driving the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate. These energy currencies are utilized for the assimilation of carbon, nitrogen and sulphur, as well as successive metabolic reactions (Eberhard et al. 2008; Baker 2008; Lawlor 2009; Ruban 2009). Figure 2.1 illustrates the process of photosynthesis.

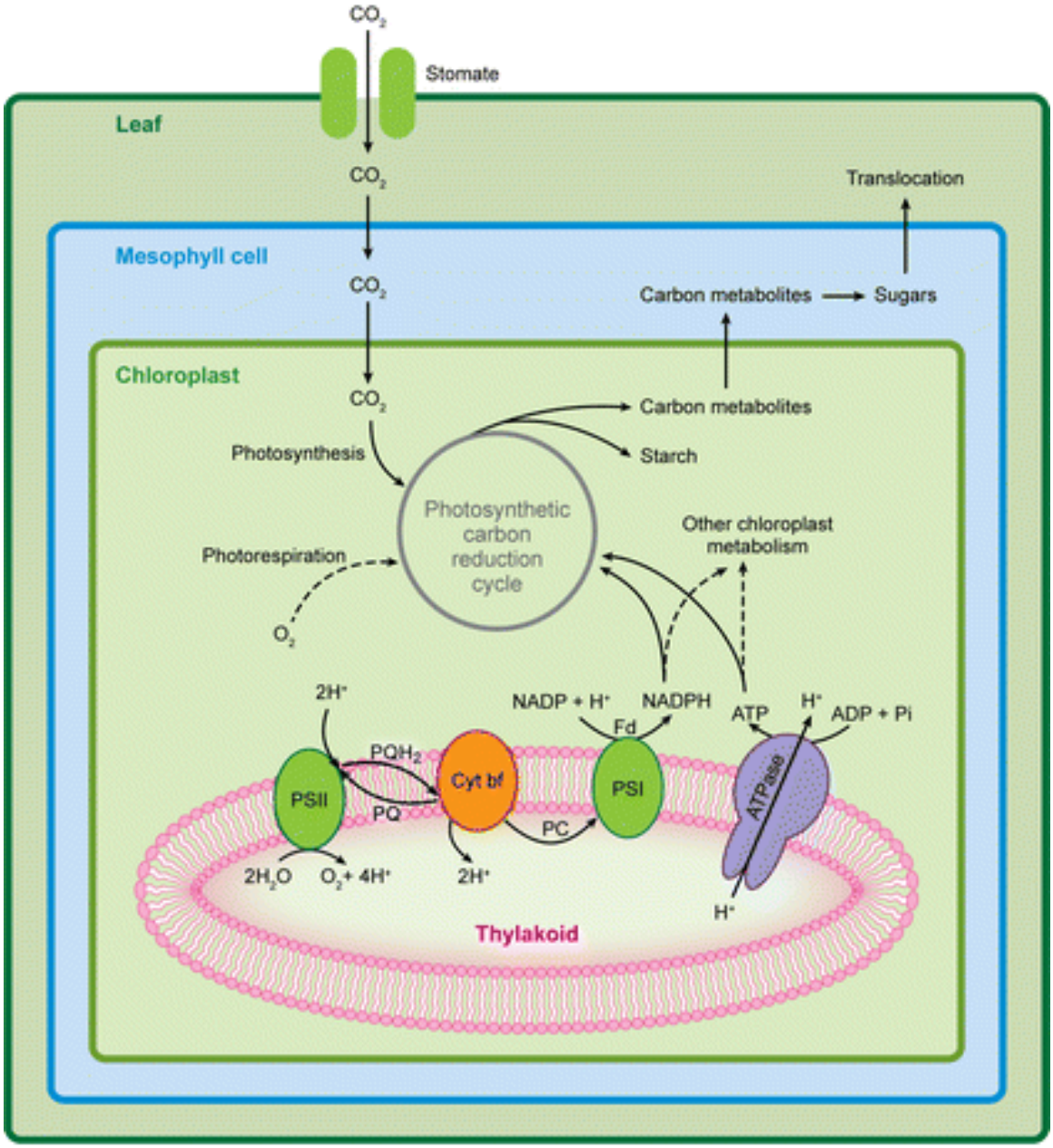


Figure 2.1. Relationships between photosynthetic electron transport, carbon metabolism and transport, and CO_2 supply (from Baker 2008). Cyt bf, cytochrome b_6f complex; Fd, ferredoxin; PC, plastocyanin; PQ, plastoquinone; PQH_2, plastoquinol; PSI, photosystem I; PSII, photosystem II; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate. Reproduced with permission of ANNUAL REVIEWS, INC: Annu Rev Plant Biol 59: 89-113, copyright (2008).

Light capture is a critical process as excitation energy can have detrimental fates. In the normal conditions, a major fraction of the light energy intercepted by the chlorophyll molecules enters into photochemistry, while rest of it escapes from plants through thermal dissipation and fluorescence (Figure 2.2). A time lag in photochemical transformation of the energy in the PSII may result in the transfer of excitation energy from chlorophyll to oxygen, producing highly reactive singlet oxygen. Similarly, unavailability of $NADP^+$ to accept the electrons from PSI in electron transport chain may result in the reduction of oxygen to form the superoxide radical or the transfer of electrons to water to form the hydroxyl radical and hydrogen peroxide. These compounds are known as ROS and have important role in cellular signalling. However, ROS also possess the potential to cause oxidative damage to nucleic acids, proteins and lipids (photodamage) (Demmig-Adams and Adams III, 2000; Eberhard et al. 2008; Lawlor 2009; Ruban 2009; Cessna et al. 2010).

The kinetics of light absorption and utilization reactions differ in several orders of magnitude. Proper functioning of the system thus entails fine regulation and coordination of photosynthetic reactions through appropriate balance between the processing of light energy and the utilization of the transformed chemical energy, the state known as photostasis (Öquist and Huner 2003; Ensminger et al. 2006). The potential capacity for energy utilization is dictated by energy demand of plants that in turn depends on inherent growth potential in the given environmental conditions. Photosynthesis is optimized when various abiotic factors, such as intensity and quality of incident light, temperature, availability of water, nutrients and carbon dioxide (CO_2) are in favourable limits. Conversely, alterations in surrounding environmental factors may perturb the photostatic balance resulting in the low efficiency of light processing and assimilatory reactions, and subsequently triggering the oxidative stress (Demmig-Adams and Adams 2000; Öquist and Huner 2003; Ensminger et al. 2006; Eberhard et al. 2008; Lawlor 2009; Ruban 2009; Cessna et al. 2010).

Plants have evolved dynamic photoprotective mechanisms responding to both transient stochastic fluctuation and long-lasting changes in the environmental conditions. In fact, photosynthesis itself has been shown to act as an environmental sensor for the efficient response to the environmental cues (Öquist and Huner 2003; Ensminger et al. 2006). Pre-existing flexibilities in energy transformation enable plants to control the light interception in the short-term. Similarly, the long-term environmental cues induce further acclimatory adjustments for achieving a new state of photostasis (photosynthetic acclimation). Photosynthetic acclimation results in modulation of both light processing (source) and energy utilization (sink) capacities (Öquist and Huner 2003; Kanervo et al. 2005; Walters 2005; Ensminger et al. 2006; Eberhard et al. 2008).

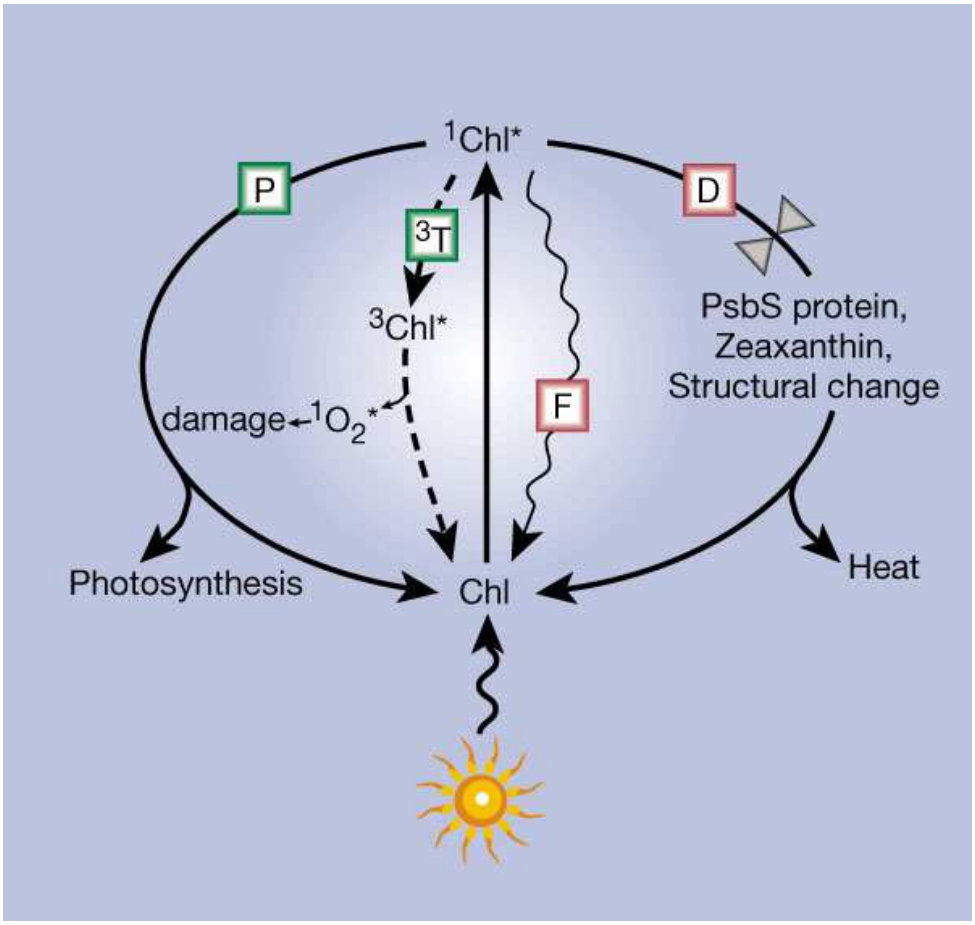


Figure 2.2. Fates of sunlight absorbed in the light-harvesting chlorophyll complexes (from Demmig-Adams and Adams III 2000). Chl, chlorophyll; ^{1}Chl*, excited singlet chlorophyll; ^{3}Chl*, excited triplet chlorophyll; P, photochemistry; D, dissipation; F, fluorescence; ^{3}T, triplet pathway; $^1O_2$*, singlet oxygen. Repinted by permission from MacMillian Publishers Ltd: Nature 403:371-374, copyright (2000).

Photosynthetic and carbon assimilation efficiencies decrease when environmental conditions or resource availability are not favourable to plants. Engagement of photoprotective mechanisms may down-regulate photochemistry, resulting in lower photosynthetic efficiencies. Similarly, oxidative damage to the photosynthetic machinery also causes a significant reduction in photosynthetic efficiency. A recent study has shown that it is ultra-violet radiation rather than photosynthetically active radiation (PAR) that causes photodamage to PSII (Takahashi and Badger 2010). The extent of decrease in photosynthetic efficiency due to photoprotective down regulation or oxidative photodamage is called photoinhibition (Demmig-Adams and Adams 2006; Ensminger et al. 2006;Takahashi and Murata 2008; Tyystjärvi 2008; Cessna et al. 2010).

The photostatic strategies in plants span across different structural and temporal hierarchies in the timescales of seconds to days. These strategies may be expressed through the intracellular to morphogenic levels in the photosynthetic organs of plants (Demmig-Adams and Adams 2006; Ruban 2009; Takahashi and Badger 2010). The mechanisms operating at the intracellular level include: *(i)* state transitions for balancing the distribution of excitation energy between PSI and PSII (Tikkanen et al. 2006, 2010); *(ii)* feed-back de-exicitation or non-photochemical quenching (NPQ) to dissipate the excess excitation energy as heat (Holt et al. 2004; 2005; Horton and Ruban 2005; Johnson and Ruban 2010); *(iii)* alternative electron transfer pathways to dissipate excess excitation energy or to balance the ATP:NADPH ratio (Cruz et al. 2005; Eberhard et al. 2008; Johnson 2010; Miyake 2010); *(iv)* turnover of fragile components (D1 protein) of PSII reaction centre involving degradation of damaged polypeptides and replacement with newly synthesized ones (Mulo et al. 2008; Nixon et al. 2010); *(v)* changes in the pigment composition to balance photochemistry and photoprotection (Gruszecki 2010; Petrov et al. 2010; Solovchenko 2010); *(vi)* increases in the capacity for scavenging the ROS (Gill and Tuteja 2010; Sirikhachornkit and Niyogi 2010); and *(vii)* chloroplast movements to modulate light interception by the thylakoid membranes (Kadota et al. 2009). Similarly, at the morphogenic level, plants adjust leaf angles (Close and Beadle 2006) and thickness (Terashima et al. 2011), and display heliotropic movement of leaves or shoots to regulate the interception of light by the plant organs (Pastenes et al. 2005).

State-transitions are mechanism of balancing the distribution of excitation energy between PSI and PSII so as to maximize the efficiency of light-harvesting at low light intensities (Mullineaux et al. 2005; Tikkanen et al. 2006; 2010). State transitions occur in the time scale of minutes in response to differential excitation of the two photosystems due to changes in the spectral composition of light. The redox state of plastoquinone (PQ) pool acts as the sensor triggering the reversible migration of the LHC proteins between the two photosystems. Over-reduction of PQ pool leads to activation of a membrane-bound kinase that

phosphorylates peripheral LHC proteins (*Lhcb1* and *Lhcb2*). The phosphorylated LHC proteins migrate from PSII to PSI. When the PQ pool resumes an oxidized state, the migrant proteins undergo dephosphyrylation and then move back from PSI to PSII. Phosphorylation of LHCII is also crucial for the control of photosynthetic gene expression. In *Arabidopsis*, a thylakoid associated, Serine-Threonine kinase, *STN7*, has been shown to be required for state transitions (Bellafiore et al. 2005; Bonardi et al. 2006). In a study on *Chlamydomonas reinhardtii*, Takahashi et al. (2006) postulated that chlorophyll binding proteins *CP26*, *CP29*, and *LhcbM5* polypeptides, which were hitherto thought to be the components of PSII, shuttle between PSI and PSII during state transitions. The reduction of antenna size of PSII due to state transitions causes a quenching in the fluorescence yield of PSII. This is considered as component of NPQ and is expressed as state-transition quenching, q_T (Szabó et al. 2005).

NPQ is a short-term process occurring within a timescale of seconds to minutes for the thermal dissipation of excess excitation energy. High excitation pressure in PSII leads to the down-regulation of photosynthetic electron transport while increasing acidification of the thylakoid lumen. The low pH in the lumen activates the enzyme violaxanthin de-epoxidase that catalyzes the conversion of the xanthophyll carotenoid violaxanthin to zeaxanthin. The zeaxanthin thus formed absorbs excitation energy from excited chlorophyll and then dissipates it safely as heat (Holt et al. 2004, 2005; Horton and Ruban 2005; Johnson and Ruban 2010). Recently, it has been proposed that zeaxanthin acts as an allosteric activator and it is the photosystem II submit S (*PsbS*) protein which acts as a trigger for the conformational change in the light harvesting antenna of PSII during the process of NPQ (Crouchman et al. 2006). This ΔpH-dependant thermal dissipation, also known as energy dependant quenching (q_E) or feedback de-excitation, can be quantified as the quickly reversible portion of maximal PSII fluorescence quenching (Demming-Adams and Adams 2000). Plants also have various ROS scavenging mechanisms to prevent the photosynthetic apparatus from irreversible photodamage. The thermal energy dissipation and antioxidant processes are closely connected, as the xanthophyll cycle enzymes also require the antioxidant ascorbic acid (Hormaetxe et al. 2005; Logan et al. 2006). Carotenoids also scavenge the triplet excited state of chlorophyll and singlet oxygen leading to the dissipation of associated energy. This is a valuable strategy when the population of triplet chlorophyll increases due to lags in photochemistry (Frank and Brudvig 2004; Carbonera et al. 2005; Eberhard et al. 2008).

In addition to linear electron flow driven by PSII and PSI that function in series, there are other alternative electron transport pathways in the photosynthetic apparatus. These pathways operate over short time-scales and play important roles in plant stress responses through the initiation of q_E for

dissipating excess light and the synthesis of ATP without reduction of $NADP^+$, thereby balancing the ATP:NADPH ratio (Cruz et al. 2005; Munekage et al. 2004; Avenson et al. 2005; Eberhard et al. 2008; Miyake 2010; Johnson 2010). In one of these pathways known as water-water cycle, the electrons released from splitting the water molecules at the oxygen evolving complex of PSII are transferred to oxygen resulting in the formation of superoxide radicals. These radicals are scavenged and converted into water through the series of reactions catalyzed by enzymes superoxide dismutase and ascorbate perioxydase (Asada 1999, 2000; Logan et al. 2006). A study by Rizhsky et al. (2003) using *Arabidopsis* knock-out mutants demonstrated that the water-water cycle is essential for photoprotection even in the absence of environmental stresses.

The process of chlororespiration is another electron transport pathway that involves the reduction of PQ by the action of a thylakoidal nicotinamide adenine dinucleotide (NADH) dehydrogenase complex (NADH DH) and oxidation of reduced PQ by a plastid terminal oxidase (PTOX) resulting in the formation of water (Bennoun 2002; Peltier and Cournac 2002; Quiles 2006). It has been shown that chlororespiration plays a photoprotective role through the oxidation of stromal reductants under high irradiance and moderate heat (Bennoun 2002; Peltier 2002; Quiles 2006). The PTOX, encoded by the immutans (*Im*) gene, is suggested to be a versatile electron sink that serves as a 'safety valve' in excess light and has a crucial function in carotenoid biosynthesis (Aluru et al. 2006). However, a recent study on *Arabidopsis* grown under both warm (25°C) and cold (5°C) conditions demonstrated that the overexpression and abundance of *Im* did not augment photoprotective capacity and had no significant effect in the flux of intersystem electrons to P_{700}^+, the oxidized reaction-centre protein of PSI during steady-state photosynthesis (Rosso et al. 2006).

A third pathway of alternative electron transfer is cyclic electron transfer driven only by PSI (Munekage et al. 2004; Breyton et al. 2006; Iwai et al. 2010). Amidst the continued debate about the modulation of cyclic electron transfer (Johnson 2005), recent studies support a dynamic model for the occurrence of linear and cyclic electron flow in C_3 plants (the plants in which the CO_2 is first fixed into a compound containing three carbon atoms before entering the Calvin cycle of photosynthesis) (Breyton et al. 2006; Joliot and Joliot 2006; Johnson 2010). These processes are proposed to occur competitively between cytochrome b_6f and feredoxin-NADP reductase for electrons carried by ferredoxin (Fd) and is regulated by the balance between the redox state of PSI acceptors and donors during photosynthesis. Kotakis et al. (2006) observed that in corticular chlorenchyma, cyclic electron transport occurs at the expense of a linear electron flow. In the cyclic model, Fd binds to the stromal side of the cytochrome b_6f

complex (plastoquinol—plastocyanin reductase) and electrons are transferred to P_{700} through the Q-cycle (Breyton et al. 2006; Joliot and Joliot 2006).

As environmental fluctuation is an inevitable reality, plants experience some degree of photodamage at all light intensities (Aro et al. 2005). However, photodamage is particularly evident when the light absorption exceeds the demand of downstream metabolic sinks and overflows NPQ capacity. Photodamage to PSII occurs in two sequential steps that include a light-induced inactivation of the oxygen-evolving complex and the inactivation of the PSII reaction center by light absorbed by chlorophyll (Ohnishi et al. 2005). Excess light not only causes direct damage to PSII, but also inhibits the repair of PSII subunits through the activities of ROS (Nishiyama et al. 2006; Murata et al. 2006). One of the components of PSII heterodimer core protein, D1 is more prone to damage due to its specific task in binding most of the cofactors required for electron transfer in PSII. Plants have a highly regulated D1 turnover mechanism that occurs on medium time scales to keep photosynthesis going under stress conditions. Photooxidative damage to the D1 protein is followed in order by reversible phosphorylation of several PSII core units, dissociation of light harvesting complex of PSII (LHCII) supercomplexes, monomerization of the PSII core complex dimmers and migration of the monomers from the granal stacks to the stromal thylakoids, partial disassembly of the PSII core monomer and ubiquitination of damaged D1 protein (Aro et al. 2005; Mulo et al. 2008; Nixon et al. 2010). Recent studies have shown that light activated kinase *STN8* is required for the phosphorylation of PSII core proteins D1, D2, and CP43 proteins, and threonine residue of position 4 (Thr-4) in the PSII sub-unit PsbH protein (Bonardi et al. 2006; Vainonen et al. 2005). Likewise, the repair process involves the *de novo* synthesis of the proteins and reassembly, dimerization, formation of LHCII supercomplexes and their photoactivation (Aro et al. 2005; Rokka et al. 2005; Mulo et al. 2008; Nixon et al. 2010).

Unlike PSII, the PSI reaction centre is less prone to photodamage. The PSI reaction centre itself is an effective dissipater of energy; however, its photosensitivity increases when ROS scavenging systems are down-regulated, such as at low temperatures (Bukhov and Carpentier 2003; Rajagopal et al. 2003; 2005). A recent study has shown that the LHCs of PSI also act as a protective shield to the reaction centre. In PSI, the LHCs are the primary target of photodamage while reaction centres mostly remain photochemically functional during photoinhibitory conditions (Alboresi et al. 2009).

When plants continue to experience altered growth conditions for relatively longer timescales, the redox signals generated during photosynthesis elicit the expression of suites of genes for adjustments in the photosynthetic machinery (Walters 2005; Ensminger et al. 2006). Goulas et al. (2006) analysed the

chloroplast proteome of *Arabidopsis* plants exposed to 5°C for varying durations and identified 43 differentially displayed proteins that participate in photosynthesis, other plastid metabolic functions, hormone biosynthesis, and stress sensing/signal transduction. Several differentially expressed genes of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.) grown under various light and temperature combinations were shown to encode chloroplastic proteins (Ndong et al. 2001). The changes in the photosynthetic machinery in the relatively long timescales are also reflected by changes in chlorophyll *a:b* ratios and often decreased quantum yields for CO_2 fixation and O_2 evolution (Ensminger et al. 2006).

2.3.4.2 Photosynthetic Acclimation to Temperature

Photosynthetic acclimation involves modulation of both the mechanism of energy transformation at sources and the capacity of electron sinks in assimilatory reactions. What makes difference between the stress tolerant and susceptible plant species is their capacity to modulate their light processing and carbon assimilating enzymes (Naidu and Long 2004). The plants acclimated to altered growth conditions have altered levels of soluble protein, ribulose-1,5-bisphosphate caboxylase/oxygenase (Rubisco), Rubisco activase and sucrose-phosphate synthase (Strand et al. 1999; Stitt and Hurry 2002; Chen et al. 2005; Pandurangam et al. 2006).

Temperature dependence of photosynthesis changes with the growth temperature. In general, plants acclimated to higher temperatures maximize their photosynthetic rate at higher temperature and vice versa. Upon exposure to moderate heat stress, plant species from warmer and colder ecological conditions differed for their temperature optimum of Rubisco activation by about 10°C, suggesting the role of Rubisco activase in limiting photosynthesis at high temperature (Salvucci and Crafts-Brandner 2004). The temperature dependence of photosynthesis is associated with intercellular CO_2 concentration, activation energy of the maximum rate of RuBP (ribulose-1,5-bisphosphate) carboxylation ($V_c$max), activation energy of the rate of RuBP regeneration (Jmax), and the ratio of Jmax to $V_c$max (Hikosaka et al. 2006; Dwyer et al. 2007). Acclimation to moderate heat results in an increase in thermotolerance through the up-regulation and/or expression of several genes encoding transcription factors, regulatory proteins, heat-shock proteins and the enzymes involved in the detoxification of harmful metabolites (Lim et al. 2006). Photosynthetic acclimation to temperature also requires adjustments in the proportions of saturated and unsaturated fatty acids in membrane lipids. Such adjustments in fatty-acid composition are thought to occur through interorganellar transfer of fatty acids (Falcone et al. 2004). Acclimation to extreme temperatures also

generally involves photoprotective responses characterized by an increased accumulation of α-tocopherol, β-carotene, and xanthophyll cycle pigments and a concomitant reduction in F_v/F_m (Hormaetxe et al. 2007).

Cold acclimation and over-wintering is a metabolically active and energetically expensive venture of plants. Plants have different mechanisms to respond to transient cold shock, and short- and long-term acclimation to cold. Adaptation and acclimation to cold environments entails diverse strategies including expression of cold-active proteins and maintenance of membrane fluidity (Morgan-Kiss et al. 2006). Scots pine (*Pinus sylvestris* L.*)* seedlings relieve the excitation pressure during photosynthetic acclimation in winter through a light-dependent regulation of reaction center content and both reaction center-based and antenna-based quenching of excess light energy (Sveshnikov et al. 2006). There is differential up- and down-regulation of myriads of genes during the process of adjustment in the altered growth conditions. Comparative analysis of gene expression profiles during cold acclimation and de-acclimation of *Arabidopsis* showed that 445 genes were up-regulated and 341 genes down-regulated during cold acclimation. Similarly in the process of de-acclimation, 292 genes were up-regulated and 320 genes down-regulated. There were mutually opposite patterns of up-regulation and down-regulation of genes associated with cold acclimation and de-acclimation (Oono et al. 2006). The acclimatory changes in the structure of the photosynthetic apparatus is the result of gene expression triggered through retrograde plastid redox signals that are ensued from excitation imbalances between PSI and PSII (Fey et al.2005). Cold acclimation of photosynthesis involves up-regulation of carbon metabolism and the suppression of photorespiration (Savitch et al. 2000), seemingly as a consequence of post-translational activation of several enzymes of the Calvin cycle and malate metabolism (Stitt and Hurry 2002; Crecelius et al. 2003).

Cold acclimation of winter rye (*Secale cereale* L.) at 4°C resulted in the stronger expression of five genes of photosynthetic metabolism, three genes of RNA and protein metabolism and the gene of the antioxidative enzyme peptide methionine sulfoxide reductase (PMSR). Conversely, the transcripts of nine genes declined during de-acclimation at 22°C of cold acclimated 4°C-grown leaves. Immunoblotting showed much higher levels of PMSR in cold acclimated than in non-acclimated leaves and the protein level declined during de-acclimation. PMSR is thought to protect proteins from photodamage (In et al. 2005). Ndong et al. (2001) identified 42 differentially expressed genes from wheat and rye grown under various temperature and irradiance regimes. Several of these genes were known to encode chloroplastic proteins such as early light-inducible proteins, transketolase, carbonic anhydrase and Mg-chelatase, whereas five of the genes had ambiguous identities. It was also observed that photosynthetic acclimation responses to low temperatures resembled acclimation to high-light. Both high-light and cold acclimation displayed a

comparable redox poise of PSII and the expression of a cold acclimation gene, *Wcs*19 (Gray et al. 1997; Ndong et al. 2001; Ensminger et al. 2006). Goulas et al. (2006) analyzed the chloroplast proteome of *Arabidopsis* plants exposed to 5°C for varying durations and identified 43 differentially displayed proteins associated with photosynthesis, other plastid metabolic functions, hormone biosynthesis, and stress sensing/signaling. Analysis of the proteome of *Arabidopsis* using DIGE technology revealed differential responses to the degree of cold stress. Proteome analysis of *Arabidopsis* plants exposed to 6°C for a week showed 22 spots (proteins) with at least 2-fold altered expression, of which 18 were increased and four were decreased, while those exposed to 10°C for the same duration had more moderate expression than observed under 6°C (Amme et al. 2006).

2.3.4.3 Photosynthetic Acclimation to Light

Fluctuation in incident light is the reality of the environment in which plants grow. In low light conditions, plants cannot generate sufficient ATP and NADPH to fuel the successive carbon assimilation reactions and photosynthesis is said to be light-limited. On the other hand, excessive light generates the chemical energy that meets or exceeds the demand of downstream metabolic sinks and photosynthesis is said to be light-saturated. The energy in excess of what plants can utilize in their metabolism spills over to generate ROS leading to photoinhibition and photodamage (Kanervo et al. 2005; Ensminger et al. 2006).

Successful acclimation to the altered light conditions involves the expression of various genes and post-transcriptional adjustment of photosynthetic machinery and the antioxidant pools (Walters 2005; Ensminger et al. 2006). The light-harvesting antenna systems of PSI ad PSII have shown to behave differently during acclimation to altered light conditions. In *Arabidopsis*, antenna stoichiometry of PSI displayed stability during acclimation across different light intensities and temperatures, whereas PSII antenna size decreased in high-light and increased in low-light. With respect to the PSII reaction centre, the stoichiometry of Lhcb1, Lhcb2 and Lhcb6 (CP24) changed while that of Lhcb4 and Lhcb5 (CP29 and CP26) remained more or less stable. In high-light acclimated plants, higher accumulation of PsbS was associated with faster activation rates of q_E (Ballottari et al. 2007). Winter rye plants acclimated under high-light at warm temperatures (800 µmol photons m^{-2} s^{-1} at 20°C) or moderate light at low temperatures (250 µmol photons $m^{-2}s^{-1}$ at 5°C) exhibited a reduction in PSII photochemistry of 66% and 64% respectively; however, there were not any measurable changes in PSI photochemistry, abundance of PSI heterodimer or intersystem electron pool sizes (Ivanov et al. 1998). This suggests that photosynthetic acclimation of the light processing machinery is mainly associated with alterations in PSII.

Pigment composition of leaves is an important factor influencing acclimation to the light environment. The xanthophylls zeaxanthin and lutein take part in energy-dependent feedback de-excitation (q_E) and antioxidative activities (Eberhard et al. 2008). It is generally believed that acclimation to high-light confers a greater q_E that ensures efficient photochemistry through the maintenance of the electron transport chain in an oxidized state (Bailey et al. 2004). However, studies with *Arabidopsis* mutants deficient in q_E alone, q_E and Z synthesis, or in q_E and Z and L synthesis showed that these plants were able to acclimate to high-light in the absence of effective q_E mechanisms, through increased levels of antioxidants like α-tocopherol and ascorbate (Golan et al. 2006). Lyreleaf sage (*Salvia lyrata* L.) plants grown under high-light coupled with water deficit conditions showed enhanced oxidation of β-carotene and α-tocopherol to α-tocopherol quinine (Munne-Bosch and Cela 2006). The implication of these observations is that when the photoprotective demand exceeded the feedback de-excitation capacity of the plants, plants acclimate to high-light conditions through enhanced antioxidative mechanism. In tropical tree legumes (*Inga sp.*), the pigment-protein complexes of the photosynthetic apparatus contain very high levels of the xanthophyll lutein-epoxide in association with the xanthophyll violaxanthin. Based on a recent study, it is proposed that lutein plays a role in photoprotection during photoacclimation through the conversion of efficient light-harvesting antennae of the shade plant into potential excitation dissipating centres (Matsubara et al. 2005). In response to photoinhibitory conditions during winter acclimation, common box (*Buxus sempervirens* L.) plants showed an increase in accumulation of red (retro-) carotenoids (eschscholtzxanthin, monoanhydroeschscholtzxanthin, anhydroeschscholtzxanthin), α-tocopherol, xanthophyll cycle pigments, and a gradual decrease on chlorophyll content with a concomitant decrease in maximal photochemical efficiency. Transfer of the photosynthetic tissues from photoinhibitory winter conditions to normal room temperature for nine days resulted in the reversal of those acclimatory processes. It is suggested that red carotenoids play a role of light filters during the winter acclimation processes (Hormaetxe et al. 2005).

Plant species differ in their capacities and mechanisms to tolerate high-light (sun) and shade environments. During the transition from saturating to limiting irradiance, soybean (*Glycine max*) and rice (*Orizya sativa*) underwent a decline in photosynthesis to a low level followed by gradual increase to a stable value, while wheat (*Triticum aestivum*) and pumpkin (*Curcibita pepo*) displayed an immediate decrease of photosynthetic rate to a stable level (Chen and Xu 2006). Comparison of the effects of high- and low irradiance on physiological efficiency of plants of various growth forms showed that low light environments produced 30-130% higher SLA with 1 to 5% lower construction costs. At the same time,

there was no apparent difference in photosynthetic capacity and respiration for the low- and high-light leaves on per unit leaf mass basis (Poorter et al. 2006). Low-light acclimated maize (*Zea mays*) plants showed an increased level of LHCII and concomitant decrease in the number of structural polypeptides of LHCI. The plants acclimated to high-light displayed higher photosynthetic efficiency through adjustment of photosynthetic machinery in both mesophyll and bundle sheath cells (Drozak et al. 2006). This shows that photosynthetic acclimation occurs from intracellular to morphological scales, encompassing light interception, processing and energy utilization strategies.

Characteristics of the habitat in which plant is evolved largely determine how plants deploy their acclimatory machinery under light stress. Distributed naturally in both open and shady habitats, *Cypripedium guttatum* has the capacity to acclimate to various light conditions. Photosynthetic acclimation in this species was associated with various biochemical changes, and alterations in chlorophyll *a/b* ratio and leaf dry mass per unit area (LMA). However, differences in growth light conditions had no significant effect on the light compensation points or light saturation points for photosynthesis (Zhang et al. 2006). In the low-light environment, the chlorina F_2 chlorophyll *b*-less barley (*Hordeum vulgare* L.) mutant displayed a reduction in functional antenna size of both PSII (by 67%) and PSI (by 21%), with significantly higher NPQ of minimal fluorescence and no apparent impairment of the utilization of absorbed light energy in PS II photochemistry, as compared to the wild-type barley. The mutant was also able to acclimate to high-light through increased levels of β-carotene and xanthophyll cycle pigments (Stroch et al. 2004). Interestingly, wild-type and the chlorina F_2 mutant of barley acclimated to either high-light or low temperature showed a 2- to 3-fold increase in NPQ without involvement of either q_E, xanthophyll cycle-mediated antenna quenching or state transitions (Ivanov et al. 2006).

Photosynthetic acclimation to various stress conditions leads to stability of the thylakoid membrane composition. Gray et al. (2005) showed that temperature and light modulate the *trans*-Δ^3-hexadecenoic acid content of phosphatidylglycerol, LHCII organization and NPQ. Compared with the wild-type, the *Arabidopsis dgd1* mutant (lacking >90% of digalactosyldiacylglycerol) exhibited a higher sensitivity to and incomplete recovery of photoinhibition of PSI, and a lower capacity to undergo state transitions. This condition results in a higher excitation pressure to the PQ pool and impaired distribution of excitation energy between the photosystems (Ivanov et al. 2006). During cold-acclimation at 5°C, the *dgd1* mutant recovered pigment-protein pools and photosynthetic function equivalent to the wild-type, but the severe *dgd1* mutant phenotype reappeared upon shifting to warm temperatures. These observations implied that

relative recovery of photosynthetic activity of cold-acclimated mutant plants resulted from stable assembly of PSI complexes in the thylakoids, owing to the temperature/lipid interactions (Hendrickson et al. 2006).

Irradiance has marked effect on leaf anatomy and thickness. Leaves developed under higher irradiance (sun leaves) are thicker or have smaller cells than the leaves developed under low irradiance (shade leaves). Thicker leaves with smaller cells have greater mesophyll surface that allow better diffusion of CO_2 for optimizing carbon fixation with available light energy (Terashima et al. 2006). Rice plants grown in higher irradiance had thicker leaves with higher light-saturated rates of photosynthesis (P_{max}), higher amounts of Rubisco, and a lower chlorophyll *a:b* ratio. Contrary to the sun leaves, the morphology and anatomy of the shade leaves are shaped for maximizing the efficiency of light harvesting. Some of the shade acclimated responses include an increase in petiole length, SLA and chlorophyll *a:b* ratios. Also characterized by a limiting light environment, submerged conditions exert similar acclimatory responses in plants (Mommer et al. 2005).

Younger leaves have greater acclimation capacity to altered light conditions than full grown leaves. The transfer of rice plants from low-light to high-light triggered acclimatory responses in young leaves with altered expression of genes encoding various light processing and carbon assimilation enzymes, while there was no change in the level of Rubisco transcripts or the amount of Rubisco protein in fully developed leaves (Murchie et al. 2005). Studies on the genetic variation in the recombinant inbred lines of *Impatiens capensis* derived from a cross between sun and shade populations showed that selection in different light environments did not lead to different responses to light (Heschel et al. 2004). These observations are the indications of the developmental dependence and complex nature of heritability of acclimatory responses to light.

Adaptation and acclimation to stress environments is generally believed to be metabolically costly. Although high-light conditions require an investment by the plants in photoprotective mechanisms, this is not always counterproductive for growth. Studies with seedlings of the tropical rainforest trees (*Calophyllum longifolium* Willd; *Tectona grandis* L. f.) under natural high-light and 48% natural shaded conditions showed that high-light stress does not impair biomass accumulation of these sun-acclimated tropical tree seedlings (Krause et al. 2006).

2.3.4.4 Photosynthetic Acclimation to Multiple Stresses

Various photoprotective mechanisms operate during photosynthetic acclimation and these include NPQ, changes in pigment composition, chloroplast movement, increases in the capacity for ROS

scavenging, and leaf movement (paraheliotropism) to avoid direct exposure to sun. The relative importance of the photoprotective strategies vary with the plant species and the nature of the stress. Paraheliotropism was found to be an important photoprotective strategy in bean (*Phaseolus vulgaris* L.) under high-light and drought stress (Pastenes et al. 2005). High-light and drought stressed rosemary (*Rosmarinus officinalis* L.) plants showed an increase in *α*-tocopherol quinone (*α*-TQ) in comparison with unstressed plants. Indicative of the stress, there was strong negative correlation between the relative efficiency of PSII photochemistry (Φ_{PSII}) and the level of *α*-TQ and xanthophyll cycle de-epoxidation (Muller et al. 2006).

Understory plants experience changes in light availability and temperature due to seasonal dynamics of overstory foliage phenology. The understory plants respond to increases in irradiance and temperature from summer to autumn due to senescence of the overstory canopy, presenting increased concentrations of xanthophyll cycle pigments and a higher chlorophyll *a:b* ratio. However, acclimation to the low temperatures following in the winter involves reduced Rubisco activity. These observations imply that leaves of understory plants are able to acclimate to seasonal changes in light and temperature by varying their photosynthetic and photoprotective functions (Katahata et al. 2005). Phototropins control and integrate a variety of responses that optimize photosynthetic performance and promote plant growth under low PAR in the natural environment (Takemiya et al. 2005).

Plants have evolved mechanisms to acclimate to interacting environmental limitations. Photosynthetic electron transport has light and temperature elasticity in that the optimum temperature for photosynthetic electron transport increases with increasing growth irradiance in the canopy. Niinemets and Valladares (2004) hypothesize that plant photosynthetic capacities deviate from the theoretical optimum values because of the interacting stresses in plant canopies and evolutionary trade-offs between leaf- and canopy-level plastic adjustments in light capture and utilization. Rice (*Oryza sativa* L.) leaves subjected to low temperature (15°C) and high irradiance (1,500 µmol photons m^{-2} s^{-1}) showed decreases in F_v/F_m, Φ_{PSII} and quantum efficiency of PSI (Φ_{PSI}), de-epoxidation state of xanthophyll cycle pigments and lower rates of CO_2 assimilation due to decreases in RuBP regeneration capacity resulting from decreases in the rate of the linear electron transport. The stress also induced the cyclic electron transport around PSI in photoinhibited leaves (Hirotsu et al. 2005).

Balanced plant metabolism requires adequate availability of essential plant nutrients. Conversely, nutrient deficiency has negative effects on plant physiological processes including photosynthesis. Under varying irradiances coupled with a deficient supply of nitrogen and phosphorus, tomato (*Lycopersicon*

esculentum Mill.) plants responded with acclimatory adjustment in light harvesting and electron transport activities. Nitrogen deficiency led to reduced photosynthesis, decreased sensitivity of CO_2 fixation to oxygen (O_2) concentration and increased levels of foliar starch. Phosphorus deficiency, on the other hand, affected the carboxylation capacity resulting in decreased starch levels and increased oxygen sensitivity of CO_2 fixation (de Groot et al. 2003). Similarly, magnesium deficient plants of *Beta vulgaris* L. displayed a photosynthetic down-regulation through down-sizing the PSII antenna and PSI reaction centres (Hermans et al. 2004).

One stress factor can trigger cross-tolerance to other stress factors in the plants. *Arabidopsis* plants grown in light environment supplied with ultraviolet B (UV-B) radiation had 30% higher non-photochemical quenching (NPQ) of chlorophyll fluorescence under saturating light and were more tolerant to a 12-day drought treatment than plants grown without UV-B, as indicated by a 2-fold higher photosynthetic rate and a 12% higher relative water content (Poulson et al. 2006). Within a given light regime, plants acclimate to the changes in temperature by increasing light utilization components relative to light harvesting components (Muller et al. 2005).

2.3.4.5 Differential Photosynthetic Acclimation Responses in Plants

There are both inter- and intra-specific differences in adaptation and acclimation mechanism of plants. *Arabidopsis thaliana* (L.) Heynh., *Digitalis purpurea* L. and *Silene dioica* (L.) Clairv. grown at 100 µmol photons m^{-2} s^{-1} acclimated to fluctuating irradiance between 100 and either 475 or 810 µmol photons m^{-2} s^{-1} in a regular cycle of 15 minutes and 3 hours for seven days, with increased maximum photosynthetic rate. However, the extent of acclimation, which involved changes in Rubisco and cytochrome f content as well as pigment content and composition, varied between the species (Yin and Johnson 2000). Hu et al. (2006) observed that greenhouse- and field-ecotype varieties of tomato (*Lycopersicon esculentum*) differed in the sensitivity of their photosynthetic apparatus to chilling under low-irradiance. In the control condition, the test varieties appeared at par for various physiological parameters. However, for the post-chilling period, greenhouse-ecotype varieties showed better acclimation performance, characterized by higher net CO_2 assimilation rates, Φ_{PSII} and q_P values compared to the field-ecotype varieties (Hu et al. 2006). Under high-light and heat stress conditions, three high-yielding wheat (*T. aestivum*) genotypes differed in their photosynthetic performances. Interesting relationships were found between PSII performance indicators and grain yield, where higher yield was associated with higher F_v'/F_o' (ratio of photochemical and non-photochemical rate constants in the light) and F_m'/F_m (ratio of the non-photochemical rate constants in the

dark and light adapted state) (Monneveux et al. 2003). Two varieties of common bean (*Phaseolus vulgaris*) grown under high-light and high-temperature conditions displayed differences in leaf anatomy, stomatal development and photosynthetic capacity. Despite these differences, those varieties showed similarity in other acclimatory features such as increased chlorophyll *a/b* ratios, decreased content of Lhcb proteins, increased xanthophyll cycle pool size, reduced chlorophyll contents and decreased leaf area (Wentworth et al. 2006). Amiard et al. (2005) compared the ability of species with differing mechanisms of phloem loading to regulate photosynthesis in response to the light environment. Upon transfer from low-light to high-light conditions, apoplastic loaders (*Pisum sativum* L. and *Spinacia oleracea* L.) displayed a higher degree of up-regulation in photosynthesis than the symplastic loaders (*Cucurbita pepo* L. and *Verbascum phoeniceum* L.), whereas foliar starch levels increased more in the later than the former. Saldana et al. (2005) compared *in situ* variation in photosynthetic capacity, dark respiration and SLA in fern species of the genus *Blechnum* across a light gradient and found significant interspecies differences in average photosynthetic capacity and dark respiration along with the significant interspecies variation in both the mean value and the plasticity of SLA to light availability. The results suggested that *Blechnum* species acclimate to light availability through the adjustment of leaf morphology (Saldana et al. 2005).

2.3.4.6 Photosynthetic Diagnostics of Plant Stress and Acclimation

Plant photosynthesis is highly sensitive to environmental factors. The photosynthetic mechanism senses environmental fluctuations and the resultant changes in the physiological status of the plant, primarily at PSI and PSII, can be determined utilizing various spectroscopic techniques. These parameters can reflect the light processing efficiency, redox poise of the electron transport chain, functioning of different pathways of electron transport and thermal dissipation of intercepted light energy (Baker et al. 2007; Baker 2008). PSII has a very sensitive mechanism for the sensing of environmental cues (Ensminger et al. 2006). Chlorophyll *a* fluorescence has been increasingly used as physiological diagnostic tool for stress monitoring and screening of tolerant plants over the past two decades. Chlorophyll fluorescence measurements in dark-adapted (without prime sign) and light-adapted (with prime sign) leaves generate various basic chlorophyll fluorescence quenching variables such as minimal fluorescence (F_o, F_o'), maximal fluorescence (F_m, F_m') and steady-state fluorescence (F') that can be used to estimate variable fluorescence (F_v, F_v', F_q'), maximum quantum efficiency of PSII photochemistry (F_v/F_m), PSII operating efficiency (F_q'/F_m' or Φ_{PSII}), PSII efficiency factor ($q_P = F_q'/F_v'$) or excitation pressure ($1-q_L$), non-radiative thermal dissipation ($NPQ = (F_m/F_m')-1$) and several other indicators of PSII performance (Roháček 2002;

Hendrickson et al. 2004; Baker et al. 2008; Kornyeyev et al. 2010). Figure 2.3 shows a protocol and typical chlorophyll fluorescence induction curve of chlorophyll fluorescence quenching analysis using modulated fluorescence. The physiological relevance of these parameters is presented in Table 2.1.

Exposure of plants to a stress condition usually causes an increase in the minimal and steady-state fluorescence values along with simultaneous decrease in maximum fluorescence values. Eventually, the stress response is manifested through decrease in the quantum efficiency of PSII photochemistry parameters, and increase in excitation pressure and thermal dissipation of energy, while acclimation reflects the sustained reversal of these effects (Gray et al. 1996; 2003; Lazar et al. 2005; Sveshnikov et al. 2006; Kornyeyev et al. 2010).

A wealth of literature exists demonstrating that chlorophyll fluorescence not only serves as a reporter of photosynthetic performance, but also as an indicator of the physiological competence of the plants (Baker and Rosenqvist 2004). Chlorophyll fluorescence parameter F_v/F_m was found useful indicator for the evaluation of food legume varieties for their heat tolerance (Srinivasan et al 1996). In chilling-sensitive plants, a sudden reversible drop in chlorophyll fluorescence occurs, which can be considered as a quantitative marker for screening chilling susceptibility in angiosperms (Larcher and Neuner 1989). The variable fluorescence parameter (F_v) was also found to be useful for assessing the chilling susceptibility of strawberry genotypes (Khanizadeh and DeEll 2001). In chilling tolerance studies on rice in Nepal, Sthapit et al. (1995) observed a strong correlation between the altitudinal genotypic clines and F_v/F_m ratios after chilling for 48 hours. The effect of chilling on light-adapted chlorophyll fluorescence parameters, such as the quantum yield of PSII and electron transport correlated well with overall photosynthesis in cucumber (Yu et al. 2002). With the application of quenching analysis of chlorophyll fluorescence in the screening of a flint and dent maize breeding population, a substantial increase (up to 31%) in the photosynthetic capacity of the hybrids between selected F3 inbreeding families was realized under suboptimal temperature conditions (Fracheboud et al. 1999). Chlorophyll fluorescence thus can be used as an efficient selection tool for improving the cold tolerance of crop species.

Similarly, the chlorophyll fluorescence-based screening techniques were found to be superior to traditional screening protocols for evaluating submergence tolerance in rice (Sarkar et al. 2004). In recent years, chlorophyll fluorescence has been routinely used as a rapid and non-destructive technique to screen submergence-tolerant rice cultivars in India (Sarkar et al. 2006). In the screening for waterlogging tolerance in lucerne (*Medicago sativa*), the F_v/F_m ratio detected significant differences between control and waterlogged plants as early as the 7th day of submergence treatment (Smethurst and Shabala 2003).

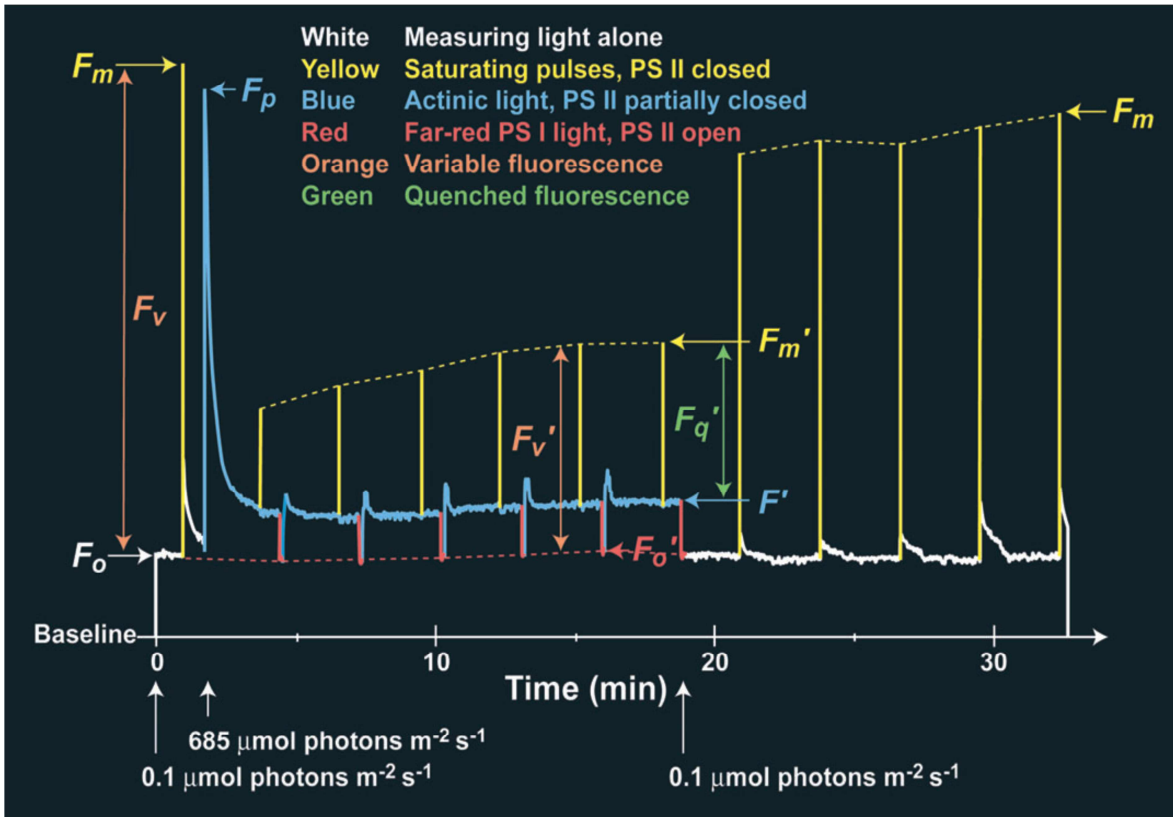


Figure 2.3. Protocol for quenching analysis using modulated fluorescence. The figure is from Baker (2008). Calculation of various quenching analysis parameters is presented in Chapter 5. F', fluorescence emission from light-adapted leaf; F_m, maximal fluorescence in the dark-adapted state; F_m', maximal fluorescence in the light-adapted state; F_o, minimal fluorescence in the dark-adapted state; F_o', minimal fluorescence in the light-adapted state; F_p, maximal fluorescence yield measured when actinic radiation is switched on; F_q', difference in fluorescence between F_m' and F' (F_m' - F'); F_v, variable fluorescence in the dark-adapted state (F_m - F_o); F_v', variable fluorescence in the light-adapted state (F_m'- F_o'). Reproduced with permission of ANNUAL REVIEWS, INC: Annu Rev Plant Biol 59: 89-113, copyright (2008).

Table 2.1 Chlorophyll fluorescence parameters frequently used in studies of PSII photochemistry (adapted from Roháček 2002; Hendrickson et al. 2004; Baker 2008)

Parameter	Definition	Physiological relevance
F, F'	Fluorescence emission from dark- or light-adapted leaf, respectively.	Provides little information on photosynthetic performance because these parameters are influenced by many factors. F' is sometimes referred to as F_s' when at steady state
F_o, F_o'	Minimal fluorescence from dark- and light-adapted leaf, respectively	Level of fluorescence when Q_A is maximally oxidized (PSII centers open)
F_m, F_m'	Maximal fluorescence from dark- and light-adapted leaf, respectively	Level of fluorescence when Q_A is maximally reduced (PSII centers closed)
F_v, F_v'	Variable fluorescence from dark- and light-adapted leaves, respectively	Demonstrates the ability of PSII to perform photochemistry (Q_A reduction)
F_q'	Difference in fluorescence between F'm and F'	Photochemical quenching of fluorescence by open PSII centers
F_v/F_m	Maximum quantum efficiency of PSII photochemistry	Maximum efficiency at which light absorbed by PSII is used for reduction of Q_A
F_q'/F_m'	PSII operating efficiency	Estimates the efficiency at which light absorbed by PSII is used for Q_A reduction. At a PPFD this parameter provides an estimate of the quantum yield of linear electron flux through PSII. This parameter has previously been termed $\Delta F/F_m'$ and Φ_{PSII} in the literature
NPQ	Non-photochemical quenching	Estimates the non-photochemical quenching from F_m to F_m'. Monitors the apparent rate constant for heat loss from PSII. Calculated from $(F_m/F_m') - 1$
q_L	Fraction of PSII centers that are 'open'	Estimates the fraction of 'open' PSII centers (with Q_A oxidized) on the basis of a lake model for the PSII photosynthetic apparatus. Given by $(F_q'/F_v')(F_o'/F')$. The expression $1- q_L$ is known as excitation pressure
q_N	Non-photochemical quenching of variable chlorophyll fluorescence	Reflects activation of the non-photochemical process during photoperiod mostly leading to the non-radiative energy dissipation to heat. Calculated as $1- (F_v'/F_v)$
q_o	Relative change of minimum chlorophyll fluorescence	Attributes to the high energy-state chlorophyll fluorescence quenching and antenna quenching. Calculated as $1-(F_o'/F_o)$
Φ_{NPQ}	Efficiency of light dependent NPQ	Refers to the fraction of light absorbed by the PSII antennae that is dissipated thermally via ΔpH and/or xanthophyll-regulated process
Φ_{NO}	Efficiency of constitutive non-photochemical energy dissipation and fluorescence	Also denoted as $\Phi_{f,D}$, it estimates the sum of the fraction of light absorbed by PSII antennae that is lost by either constitutive thermal dissipation or via fluorescence

Chlorophyll fluorescence has been used in plant screening for metal toxicity, salinity, drought, nutrient status and herbicidal effects. Moustakas et al. (1993) found chlorophyll fluorescence as a useful indicator of injury caused by aluminum toxicity in cereals. Duraes et al. (2001) found chlorophyll fluorescence as a useful tool to help screening maize germplasm for disease resistance, water-stress tolerance, aluminum toxicity tolerance and N use efficiency. Chlorophyll fluorescence measurements also detected differential responses of sunflower (*Helianthus annuus* L.) cultivars to disturbances in water availability (Germ et al. 2005). Sorghum *(Sorghum bicolor)* varieties tested under salt stress conditions demonstrate an approximate 9 and 10% decrease in F_v/F_m and q_P respectively, while electron transport rates (ETR) decreased by 20 to 25%. At the same time, NPQ increased by 44 to 50% (Netondo et al. 2004). Korres et al. (2003) demonstrated that chlorophyll fluorescence techniques could be a promising alternative to traditional methods of screening new winter wheat cultivars for their response to photosynthetic inhibitor herbicide. Fast chlorophyll fluorescence transient data analysis on chickpea showed that this technique can be used to assess the N-fixation ability (Dudeja and Chaudhary 2005). In addition, at-harvest chlorophyll fluorescence measurements of iceberg lettuce correlated with the post-harvest quality of the produce (Schofield 2005).

Chlorophyll fluorescence has also been used for screening of biotic stresses (Rolfe and Scholes 2010). Warabied and Borkowska (2004) used chlorophyll fluorescence for assessing the influence of mite (*Tetranychus urticae*) feeding on the photosynthetic apparatus of apple cultivars and found a differential tolerance of the cultivars against herbivory. Chlorophyll fluorescence imaging and thermography were found to be effective for early detection of infections by the tobacco mosaic virus (TMV) and the sugar beet fungus *Cercospora beticola* in plants (Chaerle et al. 2004, 2007).

Recently, chlorophyll fluorescence imaging, that detects and quantifies spatial variation in the F_v/F_m of the leaves, has been shown to be promising and efficient technique for the evaluation of freezing (Ehlert and Hincha 2008) and drought (Woo et al. 2008) tolerance in plants. Photochemical efficiency is at highest level when there is balance between the generation and utilization of energy, a state known as photostasis. Freezing stress destabilizes the photostatic balance leading to the photoinhibition which is reflected by the reduced photochemical efficiency of the plants (Gray et al. 2003; Ensminger et al. 2006).

While chlorophyll *a* fluorescence is an efficient tool to monitor the performance of PSII as an indicator of abiotic and abiotic stresses in plants, P_{700} spectroscopy is a useful tool to monitor the performance of PSI (Baker et al. 2007; Zhang and Sharkey 2009). PSI has crucial role in balancing the phosphorylating and reducing potential of cellular metabolism (Eberhard et al. 2008). Oxidation of P_{700} is an

indicator of PSI activity and is measured as the far-red light induced absorbance change at 820 nm ($\Delta A_{820}/A_{820}$; Harbinson and Hedley 1993; Klughammer and Schreiber 1994; Ivanov et al. 2006). The relative size of pool of electrons that can be donated to P_{700} via the intersystem chain is estimated by measuring the redox change in P_{700} caused by single- (ST) and multiple-turnover (MT) flashes of actinic light under background far-red (FR) light (Asada et al. 1992; Gray et al. 1998). A typical trace of P_{700} measurements is presented in Figure 2.4.

The exposure of the leaves to far-red (FR) light results in an absorbance change at 820 nm ($\Delta A_{820}/A_{820}$) which is an indicator of the oxidation of P_{700}, the reaction centre chlorophyll of PSI. The P_{700}^{+} is transiently reduced with the application of saturating ST flash or MT flash in the presence of background FR light. The ratio of the extent of reduction of P_{700} triggered by the MT flash to the ST flash is an indicator of the number of electrons stored in the intersystem electron transport chain or pool (e^{-}/P_{700}). Complete reduction of P_{700}^{+} to P_{700} occurrs once the FR light is turned off (Figure 2.4). The redox state of P_{700} reflects the metabolic condition of chloroplast including the availability of the electron acceptors such as $NADP^{+}$, the extent of alternative electron transfer pathways around PSI, the redox state of ferredoxin pool and electron transfer from PSII (Harbinson and Hedley 1993). These properties in turn are the function of the genotype, environmental variables and their interactions.

Other recently emerging non-invasive physiological diagnostics that hold promise to be used to help crop management strategies are near-infrared (NIR) excited Fourier transform (FT) Raman spectroscopy and multicolour fluorescence imaging. NIR-FT Raman spectroscopy has been successfully used for visualization of heterogeneous distribution of carotenoids induced by abiotic and biotic stresses (Schulz et al. 2005). For instance, this technique detected the effect of sunscald, a physiological disorder resulting from an inhibition of lycopene biosynthesis and accumulation of beta carotene in tomato fruits. Raman mapping showed a local decline of carotenoids at disease infection sites in sugarbeet (*Beta vulgaris* L.) and increases in carotenoid accumulation in response to *Septoria petroselini* infestation in parsley (*Petroselinum crispum* (Mill.) Nym.). Similarly, changes in the relative content of lutein, β-carotene and capsanthin in ripening bell pepper (*Capsicum annum* L.) were also detected by single Raman spectra (Baranski et al. 2005). Chaerle et al. (2007) observed pre-symptomatic responses to tobacco mosaic virus infection in tobacco with a multi-color fluorescence and reflectance imaging techniques. These evidences show that chlorophyll fluorescence and other imaging techniques have been very useful tools to monitor plant performance and detect stress-induced changes in plants pre-symptomatically.

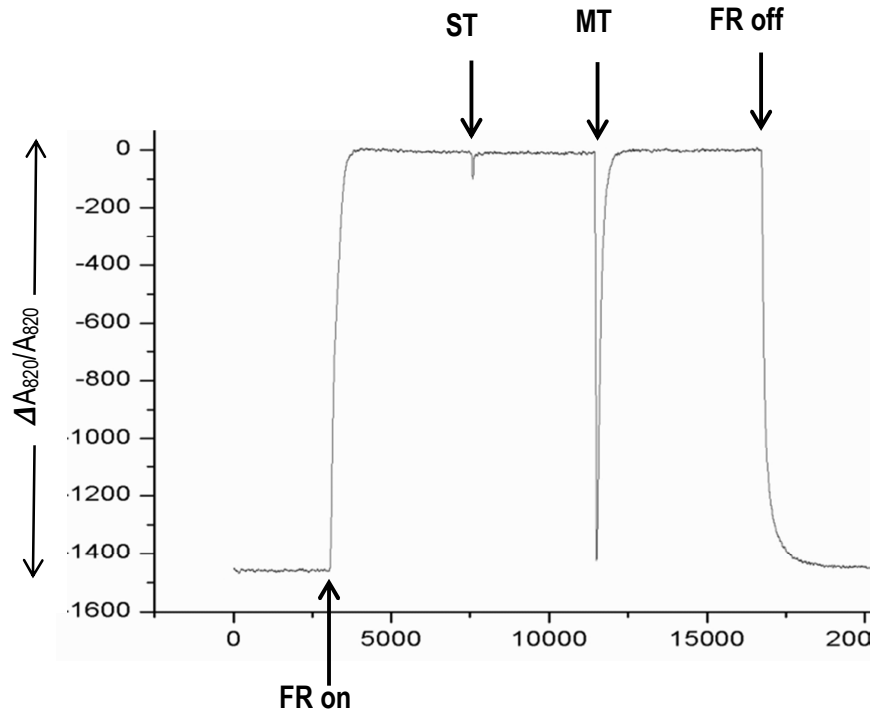


Figure 2.4. Typical trace of light-induced P_{700} transients measured as ΔA_{820}. After reaching a steady-state level of P_{700}^{+} using FR illumination, ST and MT flashes are applied. FR, far-red; MT, multiple turnover; ST, single turnover.

2.3.5 Respiratory Adjustments and Acclimation

2.3.5.1 Factors Affecting Plant Respiration

While photosynthesis is the processes of assimilating carbohydrates, respiration involves oxidative breakdown of carbohydrates for multifaceted metabolic functions. Respiration not only produces adenosine triphosphate (ATP), NADH and biosynthetic precursors, but also plays a crucial role in carbon-nitrogen interactions, optimizing photosynthesis, stress acclimation, programmed cell death, fruit ripening and thermogenesis in plants (Plaxton and Podesta 2006). Depending on the demand of the above functions, as dictated by the genetic properties and environmental conditions, daily respiratory release of CO_2 may account for as much as 70% of photosynthetically assimilated carbohydrates (Millenaar and Lambers 2003). In addition, mitochondria undergo dynamic diurnal changes in their proteomes indicating the temporal adjustment of mitochondrial functions in the day and night (Lee et al. 2010).

Various factors affect mitochondrial functionality and rates of respiration. Differential growth habits and adaptive strategies of plants may require different respiratory inputs resulting in differential respiration in plant species under varied conditions (Atkin and Day 1990; Wright et al. 2006). Usually fast growing species have higher rates of respiration and herbaceous plants have much higher specific respiration than woody plants (Poorter and Pothmann 1992; Gifford 2003). In addition, the leaves of deciduous plant species have higher dark respiration rates than those of evergreen species (Villar et al. 1995). Respiration shows a developmental dependence in that the respiration rate per unit of tissue weight decreases with increasing size and age of the plant organ (Poorter and Pothmann 1992; Villar et al. 1995; Armstrong et al. 2006a). Respiration rate remains at low level during bud dormancy and increases with growth resumption prior to budburst (Myking 1998). A study with 208 woody species at comparable physiological states in 20 different ecological settings showed that the species growing at higher irradiance or lower-rainfall sites had higher mean values of respiration and the estimated field rates of respiration were higher at warmer sites (Wright et al. 2006).

Changes in environmental factors affect plant respiration to different degrees. Because of the temperature-dependant kinetics of metabolic enzymes, change in temperature has significant effect on the plant respiration. Respiration rate increases non-linearly with the instantaneous change in temperature, and the temperature sensitivity of respiration tends to decrease as measurement temperature is increased (Atkin and Tjoelker 2003; Atkin et al. 2005a). The temperature sensitivity of respiration is more or less conserved across plant functional groups differing in maximum potential growth rate and thus demands for ATP (Campbell et al. 2007).

Most plant species show a decline in respiration under dehydration stress (Galmés et al. 2007; Atkin and Macherel 2009). Wheat (*Triticum aestivum* L.) genotypes differing in drought tolerance displayed a decrease in respiration under dehydration stress. The decrease in respiration was associated with the decrease in the proportion of mitochondrial area in the cell, from 7% of the total cellular space to 2% in drought sensitive and 6% in drought tolerant genotypes. Accordingly, compared to drought sensitive genotypes, the tolerant genotype maintained higher rates of respiration and photosynthesis, and displayed more rapid recovery of these processes to the control level after re-watering (Vassileva et al. 2009; 2011). Drought and herbicide treatments resulted in the decrease in respiration in pea (*Pisum sativum* L.) plants (Taylor et al. 2005). However, water-stressed soybean (*Glycine max* L.) did not show significant changes in the respiratory uptake of oxygen, but there was significant shift of electron flow from the cytochrome to alternative pathway resulting in an estimated decrease in ATP production by about 32% **(**Ribas-Carbo et al., 2005)**.** Salinity has species-specific effects on respiration. There was a decrease in respiration with the increasing salinity in wheat seedlings (Moud and Maghsoudi 2008) and barley (*Hordeum vulgare* L.) (Khosravinejad et al. 2008), while sugar-beet (*Beta vulgaris* L.) did not show significant changes due to salinity (Papp et al. 1983).

Nutrient deficiency seems to alter respiration in a nutrient-specific manner. For example, nitrogen, phosphorus, sulphur, manganese, zinc and molybdenum deficiencies resulted in reduced respiration rates, while potassium deficiency caused an increase in respiration rates of spinach (*Spinacia oleracea* L.) leaves (Bottrill et al. 1970). However, soybean foliage and developing seeds during the podfilling stage exhibited no consistent response of dark respiration to the level of potassium (Sale and Campbell 1988).

Elevated CO_2 was shown to have minor inhibitory effects on plant respiration (Gifford et al. 1985; Tjoelker et al. 2001; Bruhn et al. 2002). While the inhibitory effects of CO_2 were evident in C_3 species, the C_4 species (A plant in which the CO_2 is first fixed into a compound containing four carbon atoms before entering the Calvin cycle of photosynthesis) had no such response (Azocon-Bieto et al. 1994). However, findings of free-air CO_2 enrichment experiments have demonstrated that elevated CO_2 stimulates dark respiration via a transcriptional reprogramming of metabolism (Leakey et al. 2009). These contrasting observations may have emanated from the differences in the controlled variables such as temperature, supply of water and nutrients and duration of CO_2 enrichment treatments inducing respiratory acclimation.

2.3.5.2 Respiratory Q_{10}

Plant respiration increases instantaneously with increases in temperature. The temperature sensitivity of respiration is quantitatively expressed as the quotient factor Q_{10}, which is the proportional change in respiration with a 10°C increase in temperature (Atkin and Tjoelker 2003). The relationship between respiration and temperature is a predictable physiological property of plants, with Q_{10} values varying around 2 (Larigauderie and Koner 1995; Gifford 2003; Atkin et al. 2005a; 2005b). The concept of Q_{10} has also been scaled up and used as an indicator of the sensitivity of terrestrial ecosystem respiration to air temperature. A recent finding suggests a global convergence in the temperature sensitivity of respiration at the ecosystem level with Q_{10} values confined around 1.4 ± 1 across biomes without any effect of the mean annual temperature (Mahecha et al. 2010).

While the respiratory Q_{10} value of around 2 is assumed to be a standard reference in plants, various studies have demonstrated a much wider range of Q_{10} values. The recorded Q_{10} values for leaves vary from 1.4 to 4.2 and for roots from 1.1 to 4.6 (Atkin et al. 2005b). It is now well accepted that the temperature response curve of respiration is non-linear and different ranges of temperature selected for Q_{10} calculations may result in discrepancies of the resultant values. Moreover, differences in controlled experimental variables such as plant nutrient and water regimes, season and other biotic and abiotic factors affecting plant respiration may result in wider inter- and intra-specific variation in the Q_{10} values across different experiments (see Atkin et al. 2005b for review). In fact, the Q_{10} values of several plant species derived from common ranges of temperature have been found to converge to a value close to 2. For example, in 65 species, the Q_{10} value of 2.50 ± 0.11 was derived from the measurement temperature range with midpoint of 15°C (Tjoelker et al. 2001). In an earlier study with 34 species from temperate and arctic regions, the Q_{10} values derived from the temperature range of 10°C and 20°C remained around 2.45 ± 0.17. Similarly, a compilation of Q_{10} values of 125 species from published literature came up with a mean of 2.3 with most values lying between 2.0 and 2.5 (Larigauderie and Koner 1995). In the high mountain plant species *Ranunculus glacialis*, Nogués et al. (2006) observed a high rate of dark respiration with a Q_{10} of about 2 in the temperature range of 10°C and 18°C. These data suggest that the respiratory Q_{10} in a given range of measurement temperature and similar resource regimes is comparable among plant species.

Like several physiological processes, plant respiration also acclimates to altered growth temperatures. The thermal acclimation of respiration may result in a change in the temperature sensitivity of respiration, leading to a shift in the respiratory temperature response curve. Plant species exhibit

different types of respiratory responses due to cold acclimation. In some instances, cold acclimation results in a change in slope of the temperature response curve without a change in the intercept. This means that cold acclimated plants exhibit an increase in the rate of respiration only at higher measurement temperatures. This results in the change in the Q_{10} value due to acclimation. In some other instances, cold acclimation leads to the change in the intercept and an entire shift in the respiratory temperature response curve. This means that there is increase in the rate of respiration over a wide range of temperature due to cold acclimation (Atkin and Tjoelker 2003; Atkin et al. 2005a; 2005b). Plants acclimated to contrasting temperatures exhibit some degree of convergence in the rates of respiration at their respective temperatures. This condition is known as respiratory homeostasis (Tjoelker et al. 1999; Atkin and Tjoelker 2003; Atkin et al. 2005a; 2005b). *Arabidopsis* leaves developed entirely at warm and cold temperatures displayed the property of respiratory homeostasis (Armstrong et al. 2006b). In a recent study, it was shown that the homeostasis of leaf respiration and photosynthesis is better in cold tolerant species than in those that are cold susceptible (Yamori et al. 2009).

2.3.6 Photosynthetic and Respiratory Cross-Talk

Photosynthesis and respiration are highly coordinated and interacting processes in plant metabolism. These processes are highly sensitive to environmental fluctuations and prone to oxidative stress. Plants possess diverse metabolic flexibilities and safety valves that enable them to minimize the risk of oxidative stress and thereby optimizing physiological performance (Plaxton and Podesta 2006; Plaxton 2010; Kramer and Evans 2011). Metabolic activities are tightly regulated through interograde, retrograde signalling and inter-organnellar metabolic signalling mechanisms. Cellular and organelle redox state, ROS, adenylates and intra-cellular metabolic pools serve as the source of signals for inter-organelle communication (Rhoads and Subbaiah 2007; Woodson and Chory 2008; Jung and Chory 2010; Nunes-Nesi et al. 2010). The interactions between chloroplastic and mitochondrial processes can lead to the activation of appropriate pre-existing metabolic by-passes or induction of acclimatory mechanisms in order to minimize the risk of oxidative stress and optimize metabolic performance under fluctuating environments (Raghavendra and Parmasiree 2003; Nunes-Nesi et al. 2008).

There is growing evidence that suggests diverse roles of the mitochondrial respiratory system in optimizing photosynthesis. Plant mitochondria dynamically respond to environmental conditions through the diverse functional flexibility associated with the mitochondrial tricarboxylic acid (TCA) cycle (Sweetlove et al. 2010) and electron transport chain (Noguchi and Yoshida 2008; Rasmusson and Møller 2011). These

flexibilities enable mitochondria to support the chloroplast in optimizing carbon assimilation, dissipating excess reducing equivalents and thereby preventing photoinhibition (Raghavendra and Padmasree 2003; Noguchi and Yoshida 2008). Recent studies have shown that modifications in the mitochondrial TCA cycle or electron transport pathways can have a significant impact on photosynthesis. For example, transgenic tomato (*Lycopersicon esculentum* Mill.) plants deficient in fumarase activity had an impaired stomatal function resulting in a 50% reduction in photosynthesis (Nunes-Nesi et al. 2007). On the otherhand, transgenic tomato plants with slightly reduced mitochondrial nicotinamide adenine dinucleotide (NAD)-dependent isocitrate dehydrogenase demonstrated altered nitrate metabolism along with a slight decrease in maximum photosynthetic efficiency, reduced fruit size and yield compared to the wild type (Sienkiewicz-Porzucek et al. 2010).

Growing evidence shows that alternative, non-phosophrylating electron flow pathways are up-regulated under stress conditions and are thought to play key roles in optimizing photosynthesis. The non-phosphorylating electron flow pathways are composed of different types of alternative NAH(P)H dehydrogenases (Ndhs), the alternative oxidase (AOX) and uncoupling proteins (UCP) (Noguchi and Yoshida 2008). Light-dependent expression of the genes encoding respiratory by-pass components suggests that these by-passes support photosynthetic metabolism (Svensson and Rasmusson 2001; Escobar et al. 2004). Droughted plants of wheat showed significant decreases in respiration. The dehydration-induced decrease in total respiration was accompanied by a shift in respiration from the salicylhydroxamic acid (SHAM)-resistant cytochrome (Cyt) pathway to the potassium cyanide (KCN)-resistant SHAM-sensitive alternative pathway (Vassileva et al. 2009; 2011). All photoreceptors including phytochromes, phototropins and cryptochromes are implicated as the mediators of the expression of *AOX1*a gene in *Arabidopsis*. The seedlings of the *aox1a* mutant were more prone to photobleaching than wild-type under high-light growth conditions and etiolated seedlings of this mutant were delayed in chlorophyll development (Zhang et al. 2010). The AOX and Cyt pathways seem to have contrasting effects on the photosynthesis. Inhibition of either pathways resulted in a decrease Φ_{PSII} and the rate of photosynthetic O_2 evolution under high-light conditions (700 µmol photons m^{-2} s^{-1}). Under low-light (100 µmol photons $m^{-2}s^{-1}$) conditions, inhibition of AOX resulted in a decrease in photosynthetic rate, Φ_{PSII} and the Φ_{PSII}/Φ_{PSI} ratio (Yoshida et al. 2006). Levels of irradiance influenced the amount of AOX in both shade and sun species, where the enzyme appeared in the active, reduced form under high light and as the inactive, oxidized form under low light conditions (Noguchi et al. 2005). Bartoli et al. (2005) also observed that inhibition of AOX caused a reduction in the immediate quantum yield of PSII and photochemical

quenching in drought-stressed plants under high irradiance. *Arabidopsis* mutants defective in cyclic electron flow around PSI had higher amounts of AOX protein and a greater proportion of KCN-resistant respiration than the wild-type. High-light treatment caused an up-regulation of AOX in both wild-type plants and mutants with a simultaneous increase in the activities of enzymes needed to transport reducing equivalents in the chloroplast. This suggests the coordination between AOX and the enzymes involved in the transport the reducing equivalents for optimizing the transformation of energy in the photosynthetic machinery (Yoshida et al. 2007).

In *Arabidopsis*, stress conditions such as low temperature or a deficient supply of nitrogen leads to up-regulation of the *AOX1a* protein and KCN-resistant respiration (Watanabe et al. 2008; 2010). In the *aox1a* mutant, low temperature increased the expression of genes encoding antioxidant defenses along with a deviation of carbon and nitrogen balance in comparison to wild-type. This is an indication of perturbation of both photosynthetic and respiratory metabolism in *aox1a* mutant (Watanabe et al. 2008). Through the studies with *AOX1a* antisense mutants, overexpression lines and wild-type plants of *Arabidopsis*, AOX has been implicated for a role in maintaining the energy balance and ameliorating oxidative stress under cold and salinity conditions (Fiorani et al. 2005; Umbach et al. 2005; Smith et al. 2009). Armstrong et al. (2008) observed that low temperature exposure caused an increase the activity of AOX pathway only transiently, without much appreciable increase in AOX protein or abundance of *AOX1a* transcript. With cold acclimation, the AOX activity gradually subsided, while the amount of UCP and transcript abundance of both *UCP1* and the external NAD(P)H dehydrogenase (ND)-encoding gene *NDB2* increased significantly. These data suggest a coordinated series of roles for all the alternative non-phosphorylating bypasses of mitochondrial electron transport to maintain energetic homeostasis during exposure to low temperature (Armstrong et al. 2008).

This chapter reviewed the eccophysioligical information about the experimental taxa, *Thellungiella* - ecotypes Yukon and Shandong, and *Arabidopsis* - ecotype Columbia, used in this study. Relevant literature on plant stress physiology particularly the aspect of plant adaptation and acclimation to the environmental conditions were also reviewed. Also highlighted in the review are the aspects of plant growth analysis and use of various biophysical techniques that are relevant to this study. After setting this information base, the following sections present the results of current studies in four thematic areas namely, freezing tolerance, growth analysis, photosynthesis and respiration of the three experimental taxa.

3.0 FREEZING TOLERANCE OF *Thellungiella* AND *Arabidopsis*

3.1 Introduction

Thellungiella and *Arabidopsis* have differential ecological adaptations which have been discussed in detail in Chapter 2. Comparative studies have shown that *Thellungiella* is more tolerant than *Arabidopsis* to various abiotic stresses (Amtamann et al. 2005; Amtmann 2009). *Thellungiella* plants displayed higher tolerance to oxidative stress and constitutivly expressed a large number of abiotic and biotic-stress inducible genes when compared to *Arabidopsis* (Taji et al. 2004). Studies using the Shandong ecotype of *Thellungiella* demonstrated an effective cation selectivity and retention of higher levels of the osmolyte proline as part of the mechanism that enabled it to survive salinity shock up to 500 mM NaCl (Volcov et al. 2003; Inan et al. 2004; Kant et al. 2006; Volcov and Amtamann 2006). In contrast, *Arabidopsis* was unable to survive beyond 200 mM NaCl (Bressan at al. 2001; Inan et al. 2004). In comparison to non-acclimated controls, cold-acclimated Shandong rosette leaves had increased abundance of some sixty-six proteins associated with a variety of cellular functions, suggesting a massive genetic reprogramming during cold acclimation, similar to what has been observed in *Arabidopsis* (Gao et al. 2009). However, one-week cold acclimated plants of Shandong have been shown to survive a freezing stress of -15°C for 24 h with moderate injury, while this proved completely lethal for *Arabidopsis* (Inan et al. 2004).

Adaptation of the Yukon ecotype of *Thellungiella* to a habitat presenting simultaneous multiple stresses requires a dynamic mechanisms of stress tolerance. Yukon has been shown to have remarkable level of innate freezing tolerance, presenting a LT_{50} of -13°C without cold acclimation and survival to -21°C upon prior exposure to drought followed by cold acclimation (Griffith et al. 2007). In contrast, LT_{50} values for non-acclimated and cold-acclimated *Arabidopsis* plants are -3°C and -10°C respectively (Gilmour et al. 1988). Interestingly, both Yukon and *Arabidopsis* exhibit a freezing avoidance strategy through supercooling (Reyes-Díaz et al. 2006; Griffith et al. 2007). Cold acclimation of Yukon plants also triggers the expression of cold regulated genes of the *CBF* regulon, as was found in *Arabidopsis* (Chinnusamy et al. 2007; Griffith et al. 2007).

The assessment of freezing tolerance is very crucial step in the selection of parents and evaluation of progenies in crop improvement programs. Identification of plants with exceptional freezing tolerance helps to gain insight about freezing tolerance mechanisms and thereby opens the avenues of transfer of the traits to target plants. Freezing tolerance of plants can be assessed by using one or more indicators of physiological effects caused by freezing on plants. Disruptions of cellular membranes, photosynthetic

impairment or death of plants are outwardly detectable effects of freezing (Beck et al. 2007; Reulland et al. 2009). Membrane disruption leads to the leakage of cellular contents which can be detected by electrical conductivity tests (Burr et al. 1990; Verslues et al. 2006). The ultimate effect of a stress, such as freezing, can be determined by assessing if plants can regenerate after exposure to the stress (freezing temperatures). This is determined by regrowth after releasing the stress (Warren et al. 1996; Verslues et al. 2006). Both the regrowth assay and electrical conductivity tests are two widely used methods in freezing tolerance studies (Burr et al. 1990; Warren et al. 1996; Verslues et al. 2006). The major drawbacks associated with these conventional methods is that they are time-consuming (Ehlert and Hincha 2008; Woo et al. 2008). As photosynthetic impairment is one of the several effects of freezing, the severity of this stress can potentially be quantified by monitoring the maximum quantum efficiency of photosystem II (PSII) photochemistry (F_v/F_m) of the plants. This approach seems to be suitable for the plants like *Thellungiella* and *Arabidopsis* for the fact that the rosette leaves, the photosynthesizing organs, constitute virtually the entire shoot biomass of these plants. Recently, chlorophyll fluorescence imaging of F_v/F_m in frozen leaves has been shown to be a promising and efficient technique for the evaluation of freezing and drought tolerance in plants (Ehlert and Hincha 2008; Woo et al. 2008).

This chapter presents the results of freezing tolerance studies comparing two contrasting ecotypes of *Thellungiella* and *Arabidopsis*. It was hypothesized that given the contrasting ecophysiological background of these species, *Thellungiella* would possess a higher level of freezing tolerance than *Arabidopsis*. The results show that *Thellungiella* and *Arabidopsis* do not differ significantly in basal freezing tolerance, but *Thellungiella* remarkably outperforms *Arabidopsis* in acclimation capacity. In addition, a significant relationship was found between the trend of F_v/F_m derived from chlorophyll fluorescence imaging and plant survival as a function of freezing temperature.

3.2 Materials and Methods

3.2.1 Plant Material and Growth Conditions

Seeds of *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia, Col-0, stock no. CS60000) and *Thellungiella salsuginea* (Pall.) O.E. Schulz (Shandong ecotype, stock no. CS22504 and Yukon ecotype, stock no. CS22664) obtained from Arabidopsis Biological Resource Centre (ABRC, The Ohio State University, Columbus, OH, USA) were germinated in 36-cell plastic tray flats containing Sungrow Sunshine LG3 Mix Germinating soil medium (Sun Gro Horticulture Inc., Vancouver, BC, Canada) and grown in controlled environment chambers (Conviron Model PGR15; Controlled Environments Ltd., Winnipeg,

Manitoba, Canada) in the University of Saskatchewan Phytotron. After the appearance of 3rd-4th pair of rosette leaves (10-12 days after sowing), the seedlings were thinned to a final density of 2-3 seedlings per cell. Growth conditions were those previously established for the Yukon ecotype of *Thellungiella* (Griffith et al. 2007). Non-acclimated plants were grown for 3 to 4 weeks at a day/night temperature of 22/10°C with a photoperiod of 21 h and photosynthetic photon flux density (PPFD) of 250 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by fluorescent tubes (Cool White, 160 W, F72T12/CW/ VHO; Osram Sylvania Ltd, Mississauga, ON, Canada) which was determined at leaf level with a light meter (model LI-250; Li-Cor, Lincoln, NB, USA) and quantum sensor (model LI-190SA; Li-Cor). For cold acclimation, a separate flat of plants maintained under these conditions was shifted to a 5/4°C (day/night) temperatures with the same irradiance and photoperiod as the non-acclimated plants for 1 to 4 weeks for different lengths of cold acclimation. All flats were irrigated regularly with nutrient solution (Somerville and Ogren 1982) and deionized water on every alternate day.

For irradiance experiments, non-acclimated plants were grown as described above with a day/night temperature of 20/20°C and a photoperiod of 16 h. Irradiance was adjusted to 150, 250, or 350 µmol photons m^{-2} s^{-1} PPFD. For cold acclimation, a separate flat of plants maintained under these conditions was shifted to a 4/4°C (day/night) temperatures with the same irradiance and photoperiod as the non-acclimated plants as described above.

For some treatments, drought was imposed by withholding water from non-acclimated plants for approximately 4 days until the plant water content was reduced to approximately 60%. These plants were then shifted to the cold acclimation conditions described above for 1 week. Relative water content (RWC) was calculated as (FW - DW)/FW X 100%.

3.2.2 Assays of Freezing Tolerance

3.2.2.1 Freeze Test

Plants in their growth flats were subjected to 7 test temperatures separated by 5°C intervals from +5 to -25°C under minimal irradiance (0.5 µmol photons m^{-2} s^{-1}) using a controlled environment growth chamber (Conviron, Model C1112). The freezer was cooled at a linear rate of 2.5°C h^{-1} and at each 5°C interval, the plants were held for 1 hour to equilibrate. The temperature of the freezer was monitored using the temperature sensor of the chamber and the readings were verified by a digital thermometer (TruTemp 3519N, Taylor, USA) as well as normal mercury thermometer (Canlab, T2025-3G, UK). Plants were briefly misted with fine water droplets when the temperature dropped below -2°C to allow ice nucleation and

freezing. Samples were removed from the chamber once the plants remained at the selected test temperature for 1 h. They were then held overnight in the dark at 4°C in a controlled environment growth chamber (Conviron Model PGR15) to allow the samples to thaw. After 8 h of thawing, the F_v/F_m for these plants was determined by the fluorescence imaging technique (see below; Gray et al. 2003; Ehlert and Hincha 2008). The flats of plants were returned to control growth conditions for 14 d before scoring survival by regrowth (see below) and once again determining F_v/F_m ratios by imaging.

3.2.2.2 Regrowth

Freezing tolerance was determined by monitoring the regrowth of individual plants that had been frozen as described above (Section 3.2.2.1). The frozen plants were allowed to thaw for about 8 h in the dark at 4°C in a controlled environment growth chamber (Conviron Model PGR15). After having chlorophyll fluorescence imaging of the plants done, the flats of plants were then transferred back to control growth conditions to determine survival based on regrowth. Survival was defined by the appearance of new leaves after 14 d. The plant survival was expressed in terms of percentage of plants survived (number of live plants /total number of sample plants x 100%) and the sizes of plants relative to those of non-frozen treatment of +5°C. Growth analyses showed that there is moderately high correlation ($r > 0.68$) between the rosette diameter and the plant shoot biomass (see Chapter 4, Section 4.3.2). Thus, we used rosette diameter as the proxy measure of plant size in the regrowth study. A recovery index was derived as the product of the above two expressions (plant survival percentage x relative size of the plants). The measurement of plant size and derivation of the recovery index was considered important as they potentially have bearing on the plant fitness to reproduce and thereby the biomass or seed yield. LT_{50} (lethal temperature for 50% survival) was defined as the temperature at which no new rosette growth was visible in at least half of the plants.

3.2.2.3 Chlorophyll Fluorescence Imaging

Freezing tolerance was determined by monitoring F_v/F_m of the frozen plants that were used for regrowth assay. The photochemical efficiencies in control and cold acclimated plants were assessed by using chlorophyll fluorescence imaging essentially as described in Ehlert and Hincha (2008). Chlorophyll fluorescence images and numeric data were captured from whole plants at room temperature using a commercially available modulated imaging fluorometer (FluorCam; Photon System Instruments, Brno, Czech Republic) as described in detail by Gray et al. (2003). Plants were dark-adapted for 15 min prior to

measurement. The F_v/F_m ratio was calculated as $(F_m-F_o)/F_m$ according to Schreiber et al. (1994) and values integrated for the entire plant. Image data for each experiment were normalized to a false colour scale whose extremes were arbitrarily assigned values of 0 (lowest) and 0.85 (highest). This resulted in the F_v/F_m values being represented between the blue (0) and red (0.85) extremes of the false colour scale.

3.2.2.4 Electrolyte Leakage

Electrolyte leakage was compared using both non-acclimated and cold acclimated plants according to Dexter et al. (1932) as an assessment of the response to freezing. Fully grown leaf samples were harvested in a beaker containing cold (4°C) deionized water and put in styrofoam container with ice. For each sample, two to three leaves were excised, washed, rolled in damp cheesecloth and placed in a test tube containing 2 mL of deionized water in a circulating freezing bath (Thermo NESLAB, RTE 740, Portsmouth, NH, USA) equilibrated at -2°C. Each freezing treatment was replicated 3 times. Following nucleation with ice at -2°C, the samples were allowed to equilibrate until sample temperatures returned to -2°C and then cooled to the selected test temperatures between -2.5 to -25°C with 2.5 to 5°C intervals. The cooling rate of the bath was 0.5°C min^{-1}. After holding the samples for 1 h at the test temperatures, they were removed from the bath and allowed to thaw in a cold room at 4°C for 8 h. Sample temperatures were measured using a digital thermometer (TruTemp 3519N). Five mL of deionized water was added to all the tubes (including unfrozen controls kept at 4°C), followed by gentle agitation at 50 rpm on an orbital shaker (VWR S-500) at room temperature (23°C) for 4 h before being assayed for loss of electrolytes. Ion leakage was measured by conductivity as previously described with modifications (Sukumaran and Weiser 1972; Gusta and Fowler 1976). The conductivity of each sample was measured using a conductivity meter (SB20-Symphony, VWR) with a probe (G01130HT, Suhner, Switzerland).The samples were placed in a freezer at -80°C for 8 h, thawed at 4°C, shaken slowly at 23°C for 8 h, and the conductivity was measured again. Ion leakage due to freezing was calculated as a percentage and was corrected by the ion leakage of the unfrozen control.

3.2.3 Experimental Design and Data Analysis

The experiments were laid out in randomized block design, with *Arabidopsis* and the two ecotypes of *Thellungiella* as the treatments under various growth regimes. The test samples were replicated at least 3 times for electrical conductivity test and from 6 to 10 times for the regrowth assay. The relationship between the results of regrowth-based assay and chlorophyll a fluorescence imaging was evaluated by

correlation analysis using Minitab 15 (Minitab Inc., State College, PA, USA). The LT_{50} of the taxa were calculated by solving the regression equations. Various regression models including, linear, quadratic, cubic, logarithmic and binary logistic regression were tested and a model with relatively high degree of determination along with acceptable diagnostic features was adopted for different methods of freezing tests.

Whole plant survival analysis was done by using binary logistic regression with logit link function using Minitab 15 (Minitab Inc.). The Minitab output generates the parameters (Appendix A) to put in the equation of the following form:

$$Ln\ (p/1\text{-}p) = \beta_o + \beta_1 x$$

where p is the proportion of survived plants, 1-p is the proportion of dead plants, β_o and β_1 are regression coefficients, and the x is the temperature, the predictor variable of the plant survival. The whole expression in the left-hand side of the above equation is called the log-odds. The LT_{50} is the temperature at which plant survival and death are equally likely; that means the log-odds are equal to 1. So, the LT_{50} was estimated by solving the following equation:

$$\beta_o + \beta_1 x = 1$$

The model fit was very good with concordant value of over 95% resulting in the close affinity of observed and expected values of plant survival (Appendix A).

Similarly, for the electrical conductivity test, the LT_{50} was estimated as the temperature at which there was an average ion leakage of 50%. Based on the model diagnostics, both quadratic and cubic polynomial regressions satisfied the predictability of the LT_{50}. So, quadratic regression was adopted for the LT_{50} estimation for the reason of simplicity. The LT_{50} was estimated by solving the equation of the following form:

$$y = - \beta_1 x - \beta_2 x^2$$

where, y is the electrolyte leakage, x is the freezing temperature, and $\beta_1$and β_2 are regression coefficients.

3.3 Results

The Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown under various growth regimes were evaluated for their freezing tolerance in terms of LT_{50} (the temperature at which 50% of the plants are killed). In most cases, the loss of plant vitality was assayed through three methods: regrowth, chlorophyll fluorescence imaging and electrical conductivity. The regrowth LT_{50} is the temperature at which 50% of the frozen plants failed to regenerate in 14 days after releasing the stress. The temperature at

which there was a 50% leakage of cellular contents of the frozen leaves was considered the LT_{50} for electrical conductivity assay. Using chlorophyll fluorescence imaging, a trend of declining F_v/F_m as a function of the progressive freezing temperatures was established.

3.3.1 Regrowth-Based LT_{50}

The regrowth-based LT_{50} results showed that non-acclimated *Thellungiella* and *Arabidopsis* possess similar levels of freezing tolerance (Figure 3.1A and 3.2). Under the Yukon growth regime (22/10°C day/night temperature, 250 µmol photons m^{-2} s^{-2} PPFD and 21 h photoperiod), non-acclimated plants of the *Thellungiella* ecotypes and *Arabidopsis* exhibited same level of LT_{50} at -7.6°C. With the cold acclimation at 4°C for one week, all experimental taxa acquired additional but similar levels of freezing tolerance with a LT_{50} of -13°C (Figure 3.2). With the progressive increase in the period of cold acclimation, the freezing tolerance in the Yukon ecotype diverged from Shandong and *Arabidopsis*, with a linear increase in freezing tolerance reaching the maximum LT_{50} value of -22°C by three weeks of cold acclimation (Figure 3.1B and 3.2). While Shandong and *Arabidopsis* continued a similar trend of freezing tolerance until two weeks of cold acclimation with LT_{50} values of -14.9°C, Yukon outcompeted its counterparts with the LT_{50} value of -17.4°C for the same period of cold acclimation (Figure 3.2).

The length of cold acclimation time required to achieve the peak level of freezing tolerance differed for *Thellungiella* and *Arabidopsis*. While both ecotypes of *Thellungiella* continued to gain cold tolerance capacity until three weeks of cold acclimation, *Arabidopsis* reached that stage in two weeks (Figure 3.2). Prolonged acclimation caused a decrease in freezing tolerance in both *Thellungiella* and *Arabidopsis* (Figure 3.2). Both Yukon and Shandong continued with the trend of gaining cold tolerance till three weeks of cold acclimation, reaching the LT_{50} values at -22°C and -17°C respectively (Figure 3.2). With the common basal cold tolerance (LT_{50} = -7.6°C) for both *Thellungiella* and *Arabidopsis*, Yukon displayed the highest level of cold acclimation capacity (change in LT_{50} with reference to basal level) followed by Shandong. The augmentation of freezing tolerance due to cold acclimation was approximately -14°C in Yukon, -9°C in Shandong and -7°C in *Arabidopsis* (Figure 3.2). It can be inferred from these experiments that *Thellungiella* and *Arabidopsis* grown under identical non-acclimating growth conditions exhibit similar basal levels of freezing tolerance, but *Thellungiella* possess a higher cold acclimation capacity than that of *Arabidopsis*.

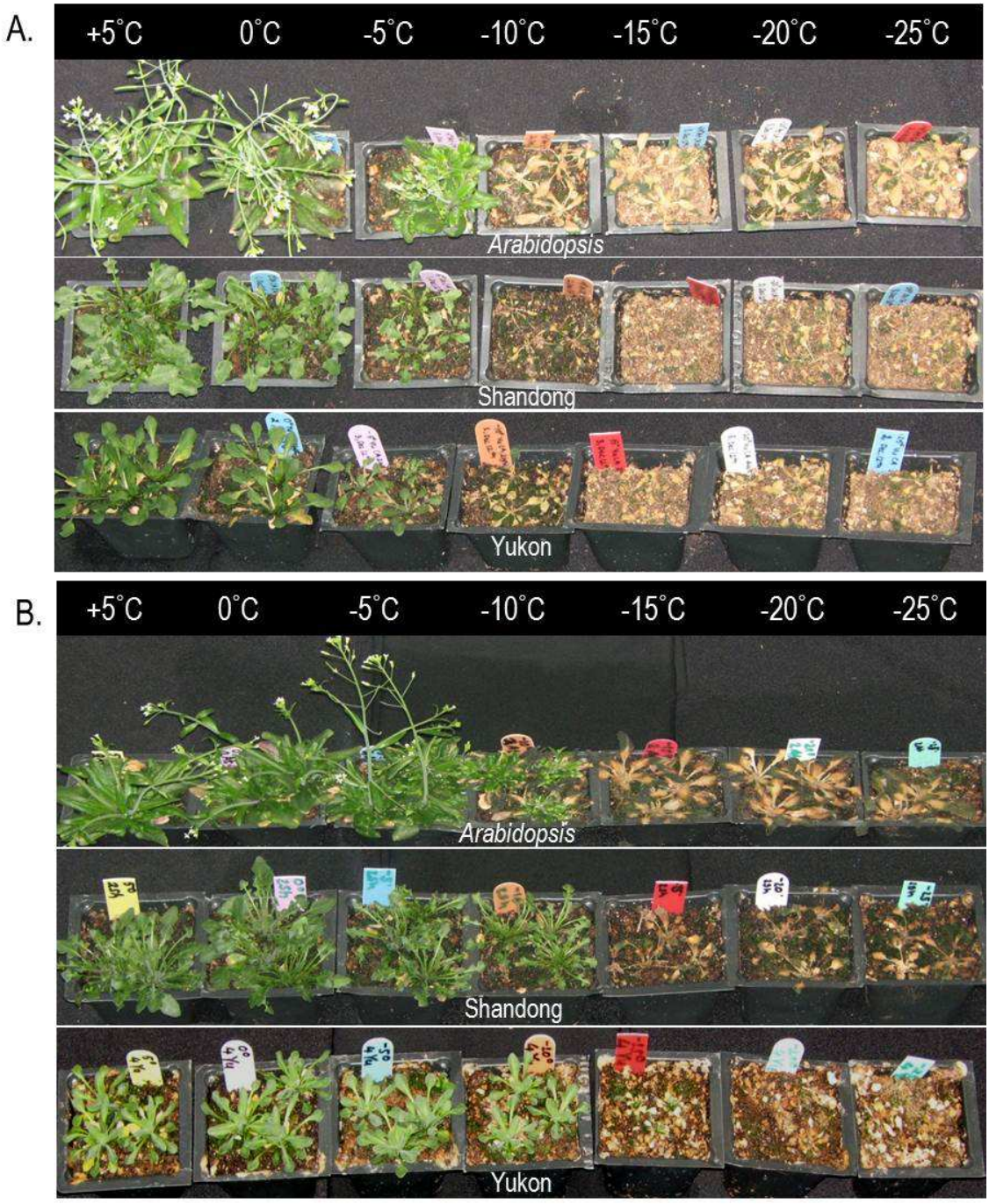


Figure 3.1. Photographs of *Thellungiella* and *Arabidopsis* plants during the regrowth assay. Plants of the Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis*, as indicated, were grown at 22/10°C (day/night) with a 21 h photoperiod and irradiance of 250 µmol photons m^{-2} s^{-1} for 4 weeks (non-acclimated) and cold acclimated for 2 weeks at 5/4°C (day/night) without varying other environmental parameters and subjected to the regrowth assay for freezing tolerance as described in Section 3.2.2. Representative photographs of non-acclimated (**A.**) and two-week cold acclimated (**B.**) plants are shown.

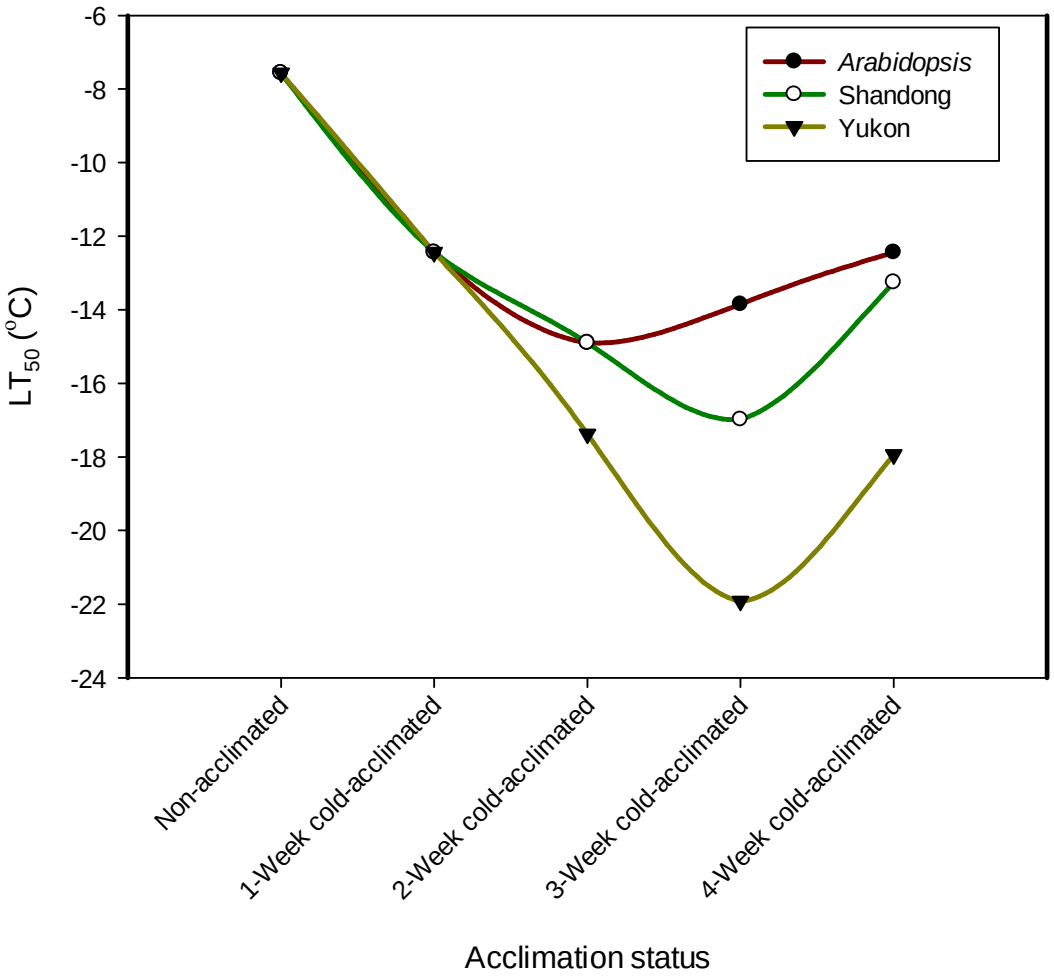


Figure 3.2. Regrowth-based LT_{50} as a function of cold acclimation time. Plants of the Yukon (●) and Shandong (○) ecotypes of *Thellungiella* and *Arabidopsis* (▼) were grown at 22/10°C (day/night) with a 21 h photoperiod and irradiance of 250 µmol photons m^{-2} s^{-1} for 4 weeks (non-acclimated) and cold acclimated for 1 to 4 weeks at 5/4°C (day/night) without varying other environmental parameters and subjected to the regrowth assay for freezing tolerance as described in Section 3.2.2. Survival percentage was calculated from 6 to 10 samples and the LT_{50} was calculated by using the parameters obtained from the binary logistic regression described in Section 3.2.3.

3.3.2 Survival Estimation Based on the Maximum Quantum Efficiency of PSII

The F_v/F_m declined progressively in a non-linear fashion with decreasing temperatures. A typical portrait of F_v/F_m against freezing temperatures is presented in Figure 3.3. The F_v/F_m values tended to decrease. At sub-zero temperatures, cold acclimated plants displayed higher F_v/F_m values than non-acclimated plants (Figure 3.3). The progressive decline in F_v/F_m with the decreasing temperature corresponds very well with the plant survival percentage determined by the regrowth assay.

The chlorophyll *a* fluorescence imaging generated false colour images showing spatial differences in the F_v/F_m values for the different parts of the plants (Figure 3.4). This helped to discern cold sensitive and cold tolerant tissues of the plants. The colour change from red through orange, yellow to green, in order, depicted the progressive decrease in F_v/F_m of the plant tissues. It is evident from Figure 3.4 that the newly appeared younger leaves and older leaves are more sensitive to cold than well-expanded young leaves. Similarly, the leaf lamina around the central rachis portion is less affected than the border region of the leaves (Figure 3.4). Comparison of the corresponding images of chlorophyll fluorescence (Figure 3.4) and those of regrowth (Figures 3.1 and 3.2) displays that the gradient of chlorophyll fluorescence image colour from red to green corresponds well with the decline in plant size and survival percentage.

The question now is if there is any threshold value of F_v/F_m that corresponds to the regrowth LT_{50} values. The relationship between the plant survival percentage and the F_v/F_m values are portrayed in a scatter plot in Figure 3.5. A critical range of F_v/F_m values better explained the nature of plant survival in these results rather than the single critical point value of F_v/F_m. Based on Figure 3.5, two distinct threshold regions of F_v/F_m were identified. One of the threshold regions lies around the F_v/F_m value of 0.4, below which there was no survival of the frozen plants. The second threshold region lies around the F_v/F_m value of 0.60, above which the frozen plants mostly survived. The region between the F_v/F_m values of 0.4 and 0.60 appeared to be the range of uncertainty; meaning plant survival or mortality is uncertain between this range of F_v/F_m values (Figure 3.5). These values may shift depending upon the plant species and the type of stress imposed during experimentation. For example, the F_v/F_m values may change depending upon whether the plant freezing and thawing is done in the dark or light, and in the later case the irradiance may amplify the effect.

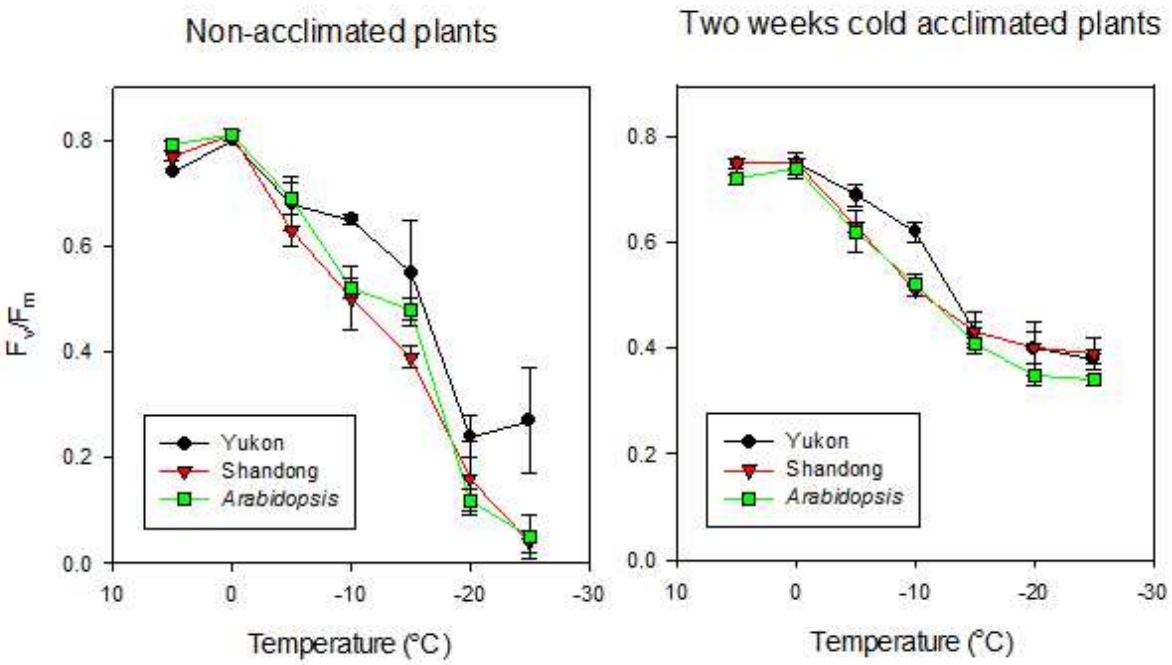


Figure 3.3. Effect of decreasing temperatures on F_v/F_m. Plants of the Yukon (●) and Shandong (O) ecotypes of *Thellungiella* and *Arabidopsis* (▼) were grown at 22/10°C (day/night) with a 21 h photoperiod and irradiance of 250 µmol photons m^{-2} s^{-1} for 4 weeks (non-acclimated) and cold acclimated for 2 weeks at 5/4°C (day/night) without varying other environmental parameters. Subsequent to the freezing test (Section 3.2.2), the level of freeze-thaw injury was assessed by chlorophyll fluorescence imaging, also described in Section 3.2.2. Representative false color chlorophyll fluorescence images are shown in Figure 3.4. Data are means ± SE, *n* = 3 to 5 plants.

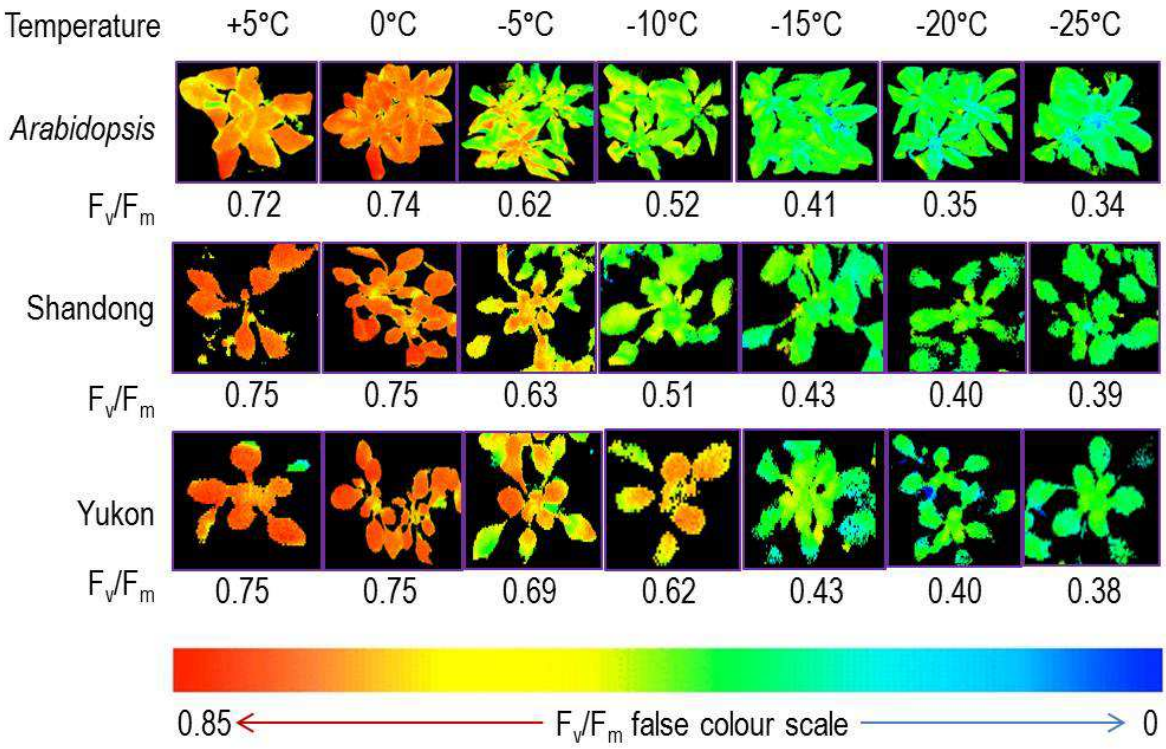


Figure 3.4. False colour images of F_v/F_m. Two week cold acclimated plants of the Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis,* as indicated, were assessed for freeze-thaw injury following the freezing test described in Section 3.2.2 by chlorophyll fluorescence imaging. Representative images are shown and were used to generate the data presented in Figure 3.3. Image data were normalized to a false colour scale whose extremes were arbitrarily assigned values of 0 (lowest) and 0.85 (highest). This resulted in the F_v/F_m values being represented between the blue and red extremes of the false colour scale.

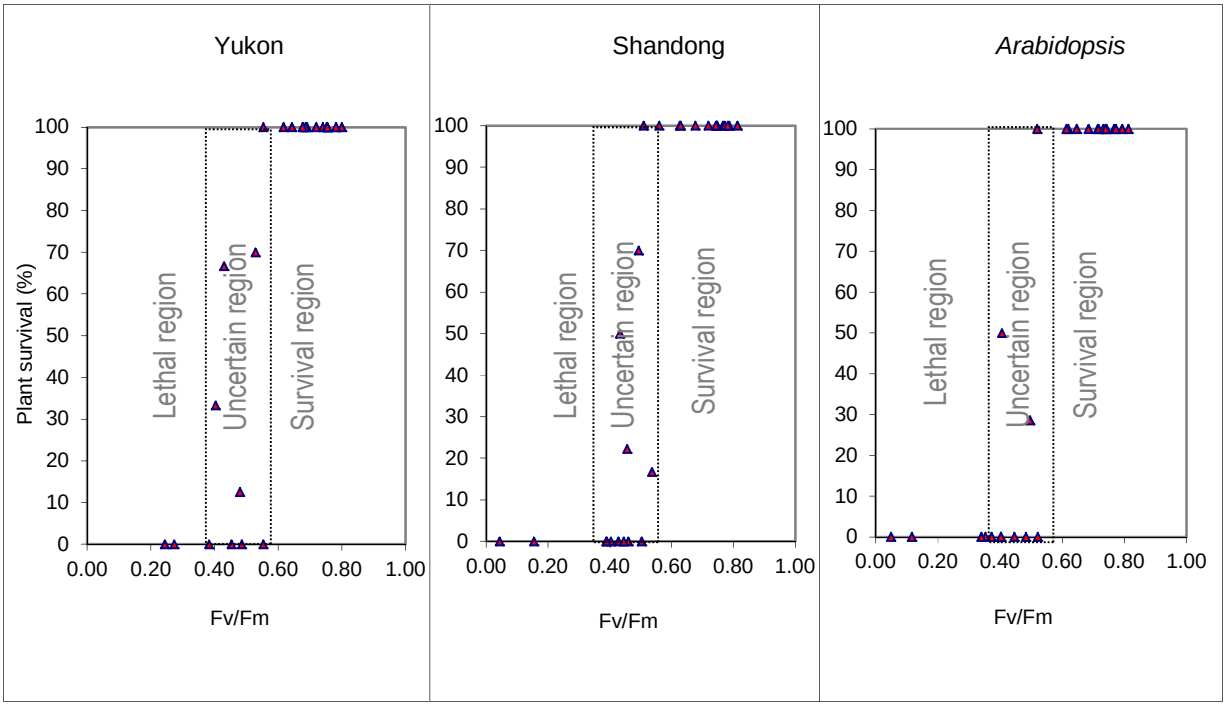


Figure 3.5. Plant survival in relation to F_v/F_m for plants of *Thellungiella* and *Arabidopsis*. Values for all three experimental taxa demonstrated similar trends within each of the threshold regions.

3.3.3 Leakage of Cellular Contents

The temperature at which the frozen leaves experienced a 50% leakage of cellular contents was considered the LT_{50} value. Based on the electrical conductivity assay, Yukon showed a higher tolerance to freezing stress under both non-acclimated and cold acclimated conditions in the Yukon growth regime (Table 3.1). The gain in cold tolerance due to cold acclimation (cold acclimation capacity) was also higher in Yukon (-7.5°C) than that of Shandong (-5°C) (Table 3.1). Compared to the corresponding LT_{50} values obtained in the regrowth assay, the electrical conductivity assay over-estimated the basal LT_{50} and under-estimated the cold acclimation capacity of the *Thellungiella* ecotypes (Table 3.1; Figure 3.2). Comparable data for *Arabidopsis* were not collected in this experiment.

3.3.4 Correlations between Regrowth and Fluorescence Imaging

Correlation analysis was done to measure the degree of correspondence between the regrowth survival scores and F_v/F_m of the plants/tissues subjected to various freezing temperatures (Table 3.1; Figures 3.2 and 3.3). Percentage of plant survival and relative size of the plants were taken as the crucial fitness parameters of the plants. We derived a third parameter and termed it as recovery index, which is simply the product of survival percentage and plant size relative to the unfrozen control plants. Obviously, these three parameters of the plant regrowth were highly correlated (r = 0.881 to 0.999) with very high degree of confidence ($P < 0.001$) (Table 3.2).

The LT_{50} values obtained through regrowth and the electrical conductivity test were compared by using a t-test. The t-test result showed that the differences between the corresponding LT_{50} values were non-significant ($P = 0.71$). In 53% of the cases, the conductivity LT_{50} slightly over-estimated the actual mortality of 50% of the plants and conversely, in the 47% of the cases, the LT_{50} obtained through regrowth assay was higher than the conductivity LT_{50}. However, the difference in the corresponding LT_{50} values was less than 1.5°C in 62% of the cases.

Our results showed that the F_v/F_m displayed by the frozen plants very well reflected the severity of stress and hence, can be used to help predict the plant survival. This is evident from the very high correlation (r = 0.87 to 0.94) between the regrowth parameters and F_v/F_m values of the plants subjected to various temperatures ranging from 5 to -25°C (Table 3.2).

Table 3.1. LT_{50} of the Yukon and Shandong ecotypes of *Thellungiella* based on electrical conductivity

Treatment	LT_{50} (°C)	
	Yukon	Shandong
Non-acclimated	-10.5	-8.0
Cold-acclimated	-18.0	-13.0

Values were calculated by using the quadratic equation as described in Section 3.2.3.
Plants were grown at 22/10°C (day/night) with a 21 h photoperiod and irradiance of 250 µmol photons m^{-2} s^{-1} for 4 weeks (non-acclimated) and cold acclimated for 3 weeks at 5/4°C (day/night) without varying other environmental parameters.

Table 3.2. Pearson's correlations between regrowth survival scores and F_v/F_m

Parameters	% of plants survived (a)	Relative size of plants (b)	Recovery index (a x b)
Relative size of plants	r = 0.883 (*P* < 0.001)		
Recovery index	r = 0.881 (*P* < 0.001)	r = 0.999 (*P* < 0.001)	
F_v/F_m	r = 0.867 (*P* < 0.001)	r = 0.941 (*P* < 0.001)	r = 0.938 (*P* < 0.001)

F_v/F_m, maximum quantum efficiency of PSII photochemistry
Correlations were performed with pooled data of all three experimental taxa subjected to the freezing assay as described in 3.2.2. Plants were grown at 22/10°C (day/night) with a 21 h photoperiod and irradiance of 250 µmol photons m^{-2} s^{-1} for 4 weeks (non-acclimated) and cold acclimated for 3 weeks at 5/4°C (day/night) without varying other environmental parameters.

3.3.5 Effects of Combined Stresses

A combination of drought followed by cold acclimation for one week further enhanced the cold tolerance in both ecotypes of *Thellungiella*. A period of drought for 4 days leading to the decrease in relative water content to approximately 60% increased the LT_{50} (increase in freezing tolerance) by 2°C in Yukon and 4°C in Shandong, with their LT_{50} values of -9.2°C and -11.6°C respectively in comparison to control values of -7.6°C (data not shown; Figure 3.2). However, there was no change in LT_{50} in *Arabidopsis* due to prior exposure to drought (data not shown). Cold acclimation of 4-day droughted plants for one week led to substantial improvement in cold tolerance in both *Thellungiella* and *Arabidopsis*. Both Yukon and Shandong possessed a higher capacity for cold acclimation under these conditions and had LT_{50} values of about -25°C, while the LT_{50} value of *Arabidopsis* reached only -13.6°C (data not shown). It may be argued that this synergistic effect of combination of drought and cold treatment might be due partly to dry soil medium that acted as the insulation to the roots which are more freezing sensitive organs of the plants. However, with no such effect observed in *Arabidopsis*, it can be concluded that *Thellungiella* ecotypes withstand multiple stress more successfully than *Arabidopsis*.

3.3.6 Influence of Light on Freezing Tolerance

Growth studies being performed in parallel to these experiments revealed a differential growth response to irradiance in *Thellungiella* and *Arabdopsis* (see Chapter 4). Thus, an experiment was designed to observe the effect of growth irradiance on freezing tolerance under non-acclimating and cold acclimating conditions. Expectedly, Yukon showed exceptionally low tolerance to freezing under low irradiance (150 µmol photons m^{-2} s^{-1}) in the non-acclimated condition (Table 3.3), suggesting that light limiting conditions rendered its physiology less competent to withstand stress. Yukon grown under 150 µmol photons m^{-2} s^{-1} had a LT_{50} value higher by 2 to 4°C as compared to those for plants grown under 250 to 350 µmol photons m^{-2} s^{-1}. Barring the exceptional case of Yukon at 150 µmol photons m^{-2} s^{-1}, there was no positive effect of increasing growth irradiance on the freezing tolerance of *Thellungiella* and *Arabidopsis* based on regrowth (Table 3.3). Unlike the results of regrowth assay where increasing irradiances did not affect the LT_{50} values, the electrical conductivity results demonstrated an increase in freezing tolerance (more negative LT_{50} values) with increasing irradiances under both non-acclimating and cold acclimating conditions (Table 3.4). However, most of the LT_{50} values from regrowth assay and electrical conductivity appeared to be quite comparable (Tables 3.3 and 3.4).

Table 3.3. Effect of irradiance on LT_{50} based on regrowth

Taxa	Non-acclimated			Cold acclimated		
	Growth irradiance (µmol photons m^{-2} s^{-1})			Growth irradiance (µmol photons m^{-2} s^{-1})		
	350	**250**	**150**	**350**	**250**	**150**
Yukon	-6.3	-6.3	-2.5	-16.6	-17.1	-17.1
Shandong	-6.3	-6.3	-6.3	-11.8	-13.1	-14.0
Arabidopsis	-6.3	-6.3	-6.3	ND	ND	-11.1

ND, not determined
Values were calculated by using the binary logistic regression equation as described in Section 3.2.3.
Plants were grown at 20/20°C (day/night) with a 16 h photoperiod at the irradiance values indicated for 4 weeks (non-acclimated) and cold acclimated for 3 weeks at 4/4°C (day/night) without varying other environmental parameters

Table 3.4. Effect of irradiance on LT_{50} based on electrical conductivity

Taxa	Non-acclimated			Cold acclimated		
	Growth irradiance (µmol photons m^{-2} s^{-1})			Growth irradiance (µmol photons m^{-2} s^{-1})		
	350	**250**	**150**	**350**	**250**	**150**
Yukon	-7.5	-6.0	-4.0	-11.0	-9.5	ND
Shandong	-8.0	-6.5	-5.0	-10.0	-9.0	-8.0
Arabidopsis	-7.5	-6.0	-4.0	-11.0	-9.0	-8.0

ND, not determined
Values were calculated by using the quadratic equation as described in Section 3.2.3
Plants were grown at 20/20°C (day/night) with a 16 h photoperiod at the irradiance values indicated for 4 weeks (non-acclimated) and cold acclimated for 3 weeks at 4/4°C (day/night) without varying other environmental parameters

3.4 Discussion

3.4.1 LT_{50}

The regrowth assay or whole-plant freeze test is a common method to assess the freezing tolerance of plants (Rietveld and Tinus 1987). The electrical conductivity test, that estimates the freeze-induced electrolyte leakage as an indicator of membrane disruption, is also a sensitive test with good predictability of changes or differences in tissue cold hardiness (Burr et al. 1990). In this study, congruence was found between regrowth-based and conductivity-based LT_{50} values. T-test comparison of LT_{50} results obtained from the two methods showed no significant difference (P = 0.71) between the LT_{50} values derived from regrowth and conductivity tests. In 53% of the cases, the conductivity LT_{50} over-estimated the actual mortality of plants (regrowth LT_{50}) and conversely, the LT_{50} obtained through regrowth assay was higher than the estimated conductivity LT_{50} in the 47% of the cases. However, the differences in the corresponding LT_{50} values under different conditions were less than 1.5°C in 62% of the cases. Similar results have been reported for the determination of freezing tolerance (LT_{50}) using the electrical conductivity test (Rietveld and Tinus 1987; Burr et al. 1990).

3.4.2 Chlorophyll Fluorescence Imaging in Freezing Tolerance Screening

Photosynthesis is highly sensitive to environmental stress and can be the source of a retrograde signal that initiates cellular stress responses (Huner et al. 1998; Ensminger et al. 2006; Fernández and Strand 2008). Analysis of cold-induced proteome of *Thellungiella* rosettes showed that nearly half of the identified cold-responsive proteins were associated with various aspects of chloroplast physiology. This suggests that the adjustments in the chloroplastic processes are an integral component of cold acclimation in *Thellungiella* (Gao et al. 2009). All stresses, including freezing cause disparity in the acquisition and utilization of photosynthetic energy and can potentially lead to photoinhibition, resulting in a decrease of F_v/F_m. Chlorophyll fluorescence measurements can efficiently detect various photosynthetic performance indicators including the F_v/F_m (Baker 2008).

Chlorophyll fluorescence has been used as a tool for measuring stress tolerance and stress-induced injuries in crops for several decades (Smillie and Hetherington 1983; Lichtenthaler and Miehe 1997; Baker and Rosenqvist 2004; Lazar et al. 2006). Recently, chlorophyll fluorescence imaging has been shown to be efficient non-invasive tool in quantifying freezing injury and drought tolerance. A high correlation was found between the F_v/F_m values and electrolyte leakage of frozen *Arabidopsis* leaves (Ehlert and Hincha, 2008). Similarly, in a separate drought related study, a high correlation was observed

between the decline in F_v/F_m, decreased plant water status and viability which demonstrated the effectiveness of chlorophyll fluorescence imaging for the quantitative estimation of drought survival (Woo et al. 2008).

In this study, a very high correlation was observed between the F_v/F_m values and survival scores of frozen plants at various freezing temperatures. The comparison of the regrowth survival and the corresponding F_v/F_m values revealed that chlorophyll fluorescence imaging is practically useful for qualitative categorization of the plant responses into three states: survival, uncertainty and lethal. Upon the standardization of the procedure with the control of non-experimental sources of variation such as light quality and irradiance during freezing and thawing and post-freezing waiting time before imaging measurements, the qualitative classification approach may have better convenience and practicality in the mass screening of crop plants for freezing tolerance.

3.4.3 Basal Freezing Tolerance and Cold Acclimation Capacity

Arabidopsis is known to be a glycophytic plant, while Yukon and Shandong are considered to be extremophiles, evolving in conditions of extreme salinity and/or cold, water stress, nutrient deficiency or mineral toxicity (Amtamann 2009). Several studies have shown that Shandong has a higher level of salinity tolerance than *Arabidopsis* and the physiological bases of differential salinity tolerance have also been elucidated (Inan 2004; Volcov et al. 2003; Volcov and Amtamann 2006; M'rah et al. 2006). Shandong was also shown to have better cold acclimation capacity than that of *Arabidopsis* (Inan et al. 2004). Cold-induced gene expression patterns in Yukon have suggested that this ecotype possess novel mechanisms of photosynthetic acclimation and responses to stress. Little overlap between the expression of cold-, salinity- and drought-induced genes, as well as the novel nature of the majority of overlapped genes, indicates that Yukon can be a good model plant for genetic and physiological studies of stress physiology (Wong et al. 2006). Griffith et al. (2007) reported that non-acclimated Yukon thrives in freezing temperatures as low as -13°C and it acquired freezing tolerance up to -21°C through a combination of cold acclimation and drought treatments. On the other hand, the control and cold acclimated values of freezing tolerance of *Arabidopsis* have been found to be approximately -6°C and -13°C respectively (Livingston et al. 2007).

This study very well corroborated the earlier findings about the level of freezing tolerance in *Arabidopsis*. The results relating to the freezing tolerance of cold acclimated *Thellungiella* are also very similar to those of earlier studies. An increasing trend in freezing tolerance with the increase in time of cold

acclimation until two weeks in *Arabidopsis* and three weeks in *Thellungiella* was noted. These observations corresponded with the results of a recent proteomic study of *Thellungiella* rosette leaves where an increase in cold acclimation time from 2 hours through 5 days to 24 days augmented the abundance of dozens of cold-responsive proteins (Gao et al. 2009). It can be hypothesized that the increase in freezing tolerance with the increasing acclimation time may be due to attainment of a better homeostatic state with the development of multiple protection mechanisms at molecular, physiological or morphological levels. However, the diminishing cold tolerance beyond 2 weeks of cold acclimation in *Arabidopsis* and three weeks of cold acclimation in Yukon and Shandong may be due to the developmental transition from the vegetative to reproductive phase which is inherently more cold susceptible.

Contrary to the expectation, non-acclimated *Thellungiella* and *Arabidopsis* displayed equivalent degrees of freezing tolerance (LT_{50} of about -7.6°C) which was close to the earlier reported value in *Arabidopsis* and much lower than that of Yukon (LT_{50} = -13°C determined by regrowth) as reported by Griffith et al. (2007). However, non-acclimated LT_{50} values were similar when determined by conductivity (Griffith et al. 2007). The divergence in freezing tolerance between the experimental taxa occurred only after two weeks of cold acclimation. The discrepancy between the results of comparable experiments may have arisen from either genetic or environmental or both factors. In the genetic aspect, it may be associated with the stress memories (Molinier et al. 2006) carried by the seed. In their study, Griffith et al. (2007) used second generation of plants from the seeds directly harvested from their natural habitat in the Yukon Territory, while the seed in the current study came through many cycles of regeneration in controlled environment chambers. Furthermore, the plant growth conditions in our experiments differed with that of Griffith et al. (2007) in light sources in that their light source was both incandescent and fluorescent bulbs. In this study, only fluorescent light was used to provide the same level of irradiance. Light quality has regulatory effect on the expression of cold-responsive transcriptional activator gene *CBF* (Franklin and Whitelam 2007) and this could have influenced our results. Another source of non-experimental variation between these comparable experiments was the supplemental nutrition. In this study, a modified Hoogland solution was applied comprising almost all mineral nutrients, while Griffith et al. (2007) fertilized with 1 g L^{-1} of 20-20-20 NPK. Comparative data analysis of *Arabidopsis* accessions with common experimental protocols revealed significant differences in the phenotypes and molecular profiles between laboratories. Those differences were attributed to the small variations in growing conditions, specially the light quality and handling of plants (Massonnet et al. 2010). These findings suggest that minor variation in the non-experimental factors may produce divergent results from the comparable experiments.

3.5 Conclusions

Thellungiella showed much higher freezing tolerance in comparison to *Arabidopsis* after two weeks of cold acclimation from the non-acclimated state. This study revealed that *Thellungiella* and *Arabidopsis* do not differ in the constitutive level of freezing tolerance and short-term cold acclimation capacity. However, non-lethal drought induces higher level of freezing tolerance in both Yukon and Shandong, but not in *Arabidopsis*. *Thellungiella* gains a much higher degree of freezing tolerance than *Arabidopsis* upon long-term cold acclimation. The combination of drought treatment and one week of cold acclimation was more effective than long-term cold acclimation in achieving maximum level of freezing tolerance in *Thellungiella*. Chlorophyll fluorescence imaging can be advantageously applied as a non-invasive freezing tolerance assay for the qualitative categorization of plant responses into survival, uncertainty and lethal states. With some refinements of the chlorophyll fluorescence imaging protocol, this qualitative classification approach may be applied to the mass screening of crop plants for freezing tolerance.

4.0 COMPARATIVE GROWTH FEATURES OF *Thellungiella* AND *Arabidopsis*

4.1 Introduction

Arabidopsis is the most extensively characterized model plant of experimental biology. Preferential use of *Arabidopsis* for plant biological studies is attributed, in part, to its growth features and life cycle (Al-Shehbaz and O'Kane 2002; Pigliucci 2002; Berardini and Rhee 2004; Bevan and Walsh 2005; Koornneef and Meinke 2010). However, the use of *Arabidopsis* for stress tolerance studies is constrained by its glycophytic adaptation with a relatively low tolerance to abiotic stresses. A search for stress-tolerant model systems among *Arabidopsis* relatives led to the identification of the extremophilic genus *Thellungiella* as a promising alternative to *Arabidopsis* (Bressan et al. 2001; Antmann et al. 2005; Amtamann 2009). Comparative taxonomy, ecophysiology and genetic attributes of *Thellungiella* and *Arabidopsis* have been reviewed in Chapter 2. A number of comparative studies between *Thellungiella* and *Arabidopsis* have attempted to probe into the genetic mechanisms governing stress tolerance (Inan et al. 2004; Griffith et al. 2007; Kant et al. 2008; Oh et al. 2010; Orshni et al. 2010).

Arabidopsis is described as an annual, facultative long-day plant with ruderal growth strategy in its natural habitat (Banta et al. 2007; Samis et al. 2008). *Arabidopsis (*ecotype Columbia) is a reference genotype because of its detailed physiological and biochemical characterization along with the availability of extensive collections of mutants, high-quality sequence and microarray data (Koornneef and Meinke 2010). *Arabidopsis* responds positively to photoperiod for reproductive transitions. Long photoperiods promote early reproductive transitions with fewer number of rosette leaves (NRL). Conversely, short photoperiods favour the production of profuse rosette leaves with a significant delay in flowering. Flowers develop acropetal order in indeterminate inflorescence (Suh et al. 2003; Samis et al. 2008). Under long-day conditions (16 h light/8 h dark), *Arabidopsis* starts flowering in 4 weeks leading to ripening of first silique in 7 weeks (Boys et al. 2001).

Thellungiella ecotypes have come under widespread experimental use in the past few years. Recently, there has been increasing use of *Thellungiella* Shandong ecotype and *Thellungiella* Yukon ecotype in stress tolerance studies. Seeds of the Yukon ecotype were recently recovered from their natural habitats of sub-arctic saline meadow where the plants grow as a summer annual with yearly variation in growth from dwarf to well-grown tall phenotypes depending on the weather pattern of the season. The natural habitat of this ecotype is characterized by a short growing season with long days, large diurnal variations of temperature, high intensity of solar radiation, soil salinity and aridity. Having evolved in

the habitat of simultaneous, multiple stresses and a short growing season, the Yukon ecotype is believed to have constitutive mechanism to tolerate extreme cold, salinity, drought, mineral toxicity or nutrient deficiency (Wong et al. 2005; Griffith et al. 2007; Kant et al. 2008; Amtamann 2009). The Shandong ecotype on the other hand is a winter annual plant adapted to the high-salinity coastal areas of north-eastern China. Reportedly, Shandong requires vernalization (long-term exposure to cold) for the reproductive transition. Shandong takes about 10 months to flower without vernalization while six weeks of low temperature (4°C) treatment is effective in triggering flowering in fully-grown plants (Inan et al. 2004; Fang et al. 2006). This ecotype is known to have extreme degree of salinity tolerance and used as a model halophyte in many recent studies (Inan et al. 2004; Taji et al. 2004; Amtmann et al. 2005; M'rah et al. 2006; Oh et al. 2010; Orsini et al. 2010; Wang et al. 2010).

Thellungiella and *Arabidopsis* have been described to have similar life history and reproductive traits and share over 90% genetic identities (Wang et al. 2004; Wong et al. 2005). However, a comparative phenotypic profile of these model systems in a reference growth regime is still lacking. In addition, the developmental prehistory of the plant has long been known to be an important factor in studies of environmental stress (Krol et al. 1984).Our understanding is still far from precise regarding the degree of resemblance/contrast between *Thellungiella* and *Arabidopsis* in their adaptive features and acclimatory responses to the environment.

This chapter presents the results of a comparative growth characterization study examining two contrasting ecotypes of *Thellungiella* and *Arabidopsis*. The results demonstrate differences in growth characteristics and developmental phenology between the plant taxa within a growth regime and within plant taxa across different growth regimes. These data highlight the importance of standardizing optimal growth conditions with proper experimental design for a better comparison of results between studies, as growth environment is likely to have a significant impact on stress responses (Krol et al. 1984).

4.2 Materials and Methods

4.2.1 Plant Material and Growth Conditions

Seeds of the Yukon and Shandong ecotypes of *Thellungiella salsuginea* (Pall.) O.E. Schulz as well as *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia, Col-0) were germinated and maintained as described in Section 3.2.1 using the growth conditions described in Table 4.1. Hereinafter, these are referred to as Yukon, Shandong and *Arabidopsis* growth regimes.

Table 4.1. Non-acclimating growth regimes used throughout this thesis

Growth Regime	Temperature (light/dark)	Photoperiod (light/dark)	PPFD (µmol photons m^{-2} s^{-1})	DAT	DPI
Yukon Growth Regime	22/10°C	21/3 h	250	20.5	18.90
Shandong Growth Regime	22/19°C	16/8 h	250	21.0	14.40
Arabidopsis Growth Regime	20/20°C	16/8 h	100	20.0	5.75

DAT, daily average temperature; DPI, daily photon irradiance; PPFD, photosynthetic photon flux density

For vernalization experiments, 35-day old plants of Yukon and Shandong *Thellungiella* were shifted to a controlled environment growth chamber with a 4/4°C (light/dark) temperature, 100 µmol photons m^{-2} s^{-1} PPFD and a photoperiod of 16 h. After 15 days the plants were shifted back to their respective growth regimes.

For irradiance experiments, plants of all three taxa were grown from seed under warm (20/20°C day/night temperature) and cold (4/4°C day/night temperature) conditions with irradiances of 100, 150, 250, 350 and 450 µmol photons m^{-2} s^{-1} PPFD and photoperiod of 16 h.

4.2.2 Growth Measurements

4.2.2.1 Absolute Growth Parameters

Absolute growth parameters including number of rosette leaves (NRL), leaf area (LA), rosette radius (RR), shoot fresh weight (FW) and shoot dry weight (DW) were measured from 3-5 individual plants. The measurements were done at 4 day intervals from 17 to 37 days after sowing (DAS) for the plants grown under Yukon, Shandong and *Arabidopsis* growth regimes. For irradiance experiments, the measurements were started at 19 DAS after sowing and repetitive measurements were taken at one week interval for the plants grown under warm (20°C) conditions, while for those grown under cold (4°C) conditions, the measurements were made at 60 DAS and repeated at two week intervals. Morphological characteristics were recorded as described by Boyes et al. (2001). The LA was measured by using LI-3100 Area Meter (LI-COR Inc. Lincoln, Nebraska, USA). DWs were obtained after drying shoots for 3 days at 70°C to constant weight. Water content was calculated as (FW-DW)/FW x 100%.

4.2.2.2 Relative Growth Parameters

Relative growth parameters were derived from the absolute growth parameters. The specific leaf area (SLA, leaf area per unit dry mass) was calculated as LA/DW. The SLA is also the reciprocal of the product of leaf thickness (LT) and leaf density and is expressed as cm^2 g^{-1} (Tholen et al. 2004; Kruger and Volin 2005). Leaf thickness (LT) was estimated as the inverse of the product of SLA and leaf dry matter content (LDMC), as established by Vile et al. (2005). Mathematically, it is expressed as $LT = (SLA \times LDMC)^{-1}$, where LDMC is the ratio of leaf dry mass to fresh mass. The unit leaf rate (ULR) and relative growth rate (RGR) were calculated on dry weight basis. ULR refers to the increase in biomass per unit leaf area per day and expressed as mg cm^{-2} d^{-1}. Likewise, RGR is the increase in dry biomass between the

periods of sampling and is expressed as RGR (mg g^{-1} d^{-1}) (Tholen et al. 2004; Kruger and Volin 2005). The mathematical expressions of RGR and ULR are as follows:

$$RGR = \{Ln(M_2) - Ln(M_1)\}/(t_2-t_1)$$

$$ULR = \{Ln(LA_2) - Ln(LA_1)\}/(LA_2-LA_1) \times (M_2-M_1)/ (t_2-t_1)$$

where M_1 and M_2 are the plant dry mass at time t_1 and t_2, respectively.

The SLA, ULR and RGR values obtained from the five consecutive time intervals were averaged to obtain the mean values for each taxon.

4.2.3 Phenological Observations

Phenological traits were recorded. These included; days to emergence of seedlings, days to appearance of rosette leaves, days to bolting, days to appearance of first flower and days to maturity of first siliqua.

4.2.4 Experimental Design and Statistical Analyses

The experiments were conducted in a completely randomized design. The growth parameters were analyzed through descriptive, correlation, regression and analysis of variance (ANOVA) techniques, using Microsoft Excel, Minitab 15 and SAS 8.1 V software. The normality of the data was graphically checked using Minitab 15 and where necessary, the data were transformed logarithmically to meet the assumptions of ANOVA. Repeated measures data were analyzed using general linear model as described by Little et al. (1998). Least significant diffference (LSD) and DMRT were used for the mean separation of one time measurements and repeated measurement data respectively.

To compare the effects of the different growth regimes the effects of temperature and light on plant growth were segregated using multiple linear regression analysis. The temperature and light variables were expressed in terms of modified growing degree days (MGDD) and daily photon irradiance (DPI) respectively. The MGDD is the cumulative daily average temperature (DAT) above the threshold temperature of 4°C and is calculated as follows:

$$MGDD= [\{(Light\ time\ temperature*photoperiod)/24 +$$
$$(dark\ time\ temperature*dark\ period)/24\} - 4\,℃] * DAS$$

For the calculation of DPI, the instantaneous units of irradiance as μmol photons m^{-2} s^{-1} were converted to mol photons m^{-2} d^{-1}, where d denotes day. Thus,

$$DPI = (\mu mol\ photons\ m^{-2}\ s^{-1})/1000000*photoperiod*60*60$$

The natural logarithmic (*Ln*) transformation of both the dependant variable (DW) and the explanatory variables (MGDD and DPI) resulted in the best fit of the model, satisfying the underlying assumptions of the regression analysis. Hence the regression model was represented by the following equation:

$$LnDW = -\beta_0 + \beta_1 LnDPI + \beta_2 LnMGDD$$

where β_0 , β_1, and β_2 are the regression coefficients.

This transformation also allows for interpretation of the regression coefficients associated with the explanatory variables. The regression coefficient associated with explanatory variable is interpreted as the percent change in the dependant variable due to a 1 percent change in the explanatory variable.

4.3 Results

4.3.1 Growth Phenology

Phenological development was faster in *Arabidopsis* followed by Yukon in all growth regimes (Table 4.2). *Arabidopsis* seedlings emerged earliest and underwent reproductive transitions in 3 to 5 weeks depending upon growth regimes. Phenological development was faster in Yukon and Shandong growth regimes which had higher DPI and day-time temperatures than that of the *Arabidopsis* growth regime. Early flowering of *Arabidopsis* in the Yukon growth regime may be attributed to the long light-period of 21 h, as this species is a facultative long-day plant.

The flowering response of *Thellungiella* was found to be very complex. Shandong flowered in about 3 months only in the *Arabidopsis* growth regime, while there was no flowering response of this ecotype in other two growth regimes without cold treatment at 4°C (vernalization). The Yukon ecotype, on the other hand, failed to undergo a reproductive transition in all three growth regimes without vernalization (Table 4.2). The plants that failed to undergo floral transitions started withering after two to three months. However, 35-day old plants of both Shandong and Yukon responded to two-weeks of vernalization and started bolting in about two months (about 10 days after shifting the cold treated plants back to warm conditions) leading to the maturity of early pods in about 3 months (Table 4.3). Yukon plants in the *Arabidopsis* regime had stagnant growth and finally collapsed without reproductive transition (Table 4.3).

In a separate study, varying irradiance with a 20°C constant temperature, the Yukon ecotype produced flowers in a short floral stalk only at higher irradiances (350 and 450 µmol photons m^{-2} s^{-1}) without vernalization treatment in about two months (Figures 4.1A and 4.2A). Shandong showed signs of flowering only at lower irradiances (100 and 150 µmol photons m^{-2} s^{-1}) without vernalization treatment in

Table 4.2. Summary of phenological events of Yukon and Shandong ecotypes of *Thellungiella* compared with *Arabidopsis* across growth regimes

Growth regime	Taxa	Days to emergence	Days to start of bolting	Days to start of flowering	Days to start of fruiting	Days to start of maturity
Yukon	Yukon	4				
	Shandong	4				
	Arabidopsis	3	20	23	25	38
Shandong	Yukon	4				
	Shandong	4				
	Arabidopsis	3	22	26	30	43
Arabidopsis	Yukon	5				
	Shandong	5	81	92	104	
	Arabidopsis	4	31	36	39	56

Table 4.3. Phenology of *Thellungiella* plants that were cold-treated at 4°C to induce flowering

Growth regime	Taxa	Days to bolting	Days to flowering	Days to fruiting	Days to first pod maturity
Arabidopsis	Yukon				
	Shandong	71	80	92	109
Yukon	Yukon	60	68	70	84
	Shandong	60	70	73	86
Shandong	Yukon	62	69	73	87
	Shandong	62	71	76	89

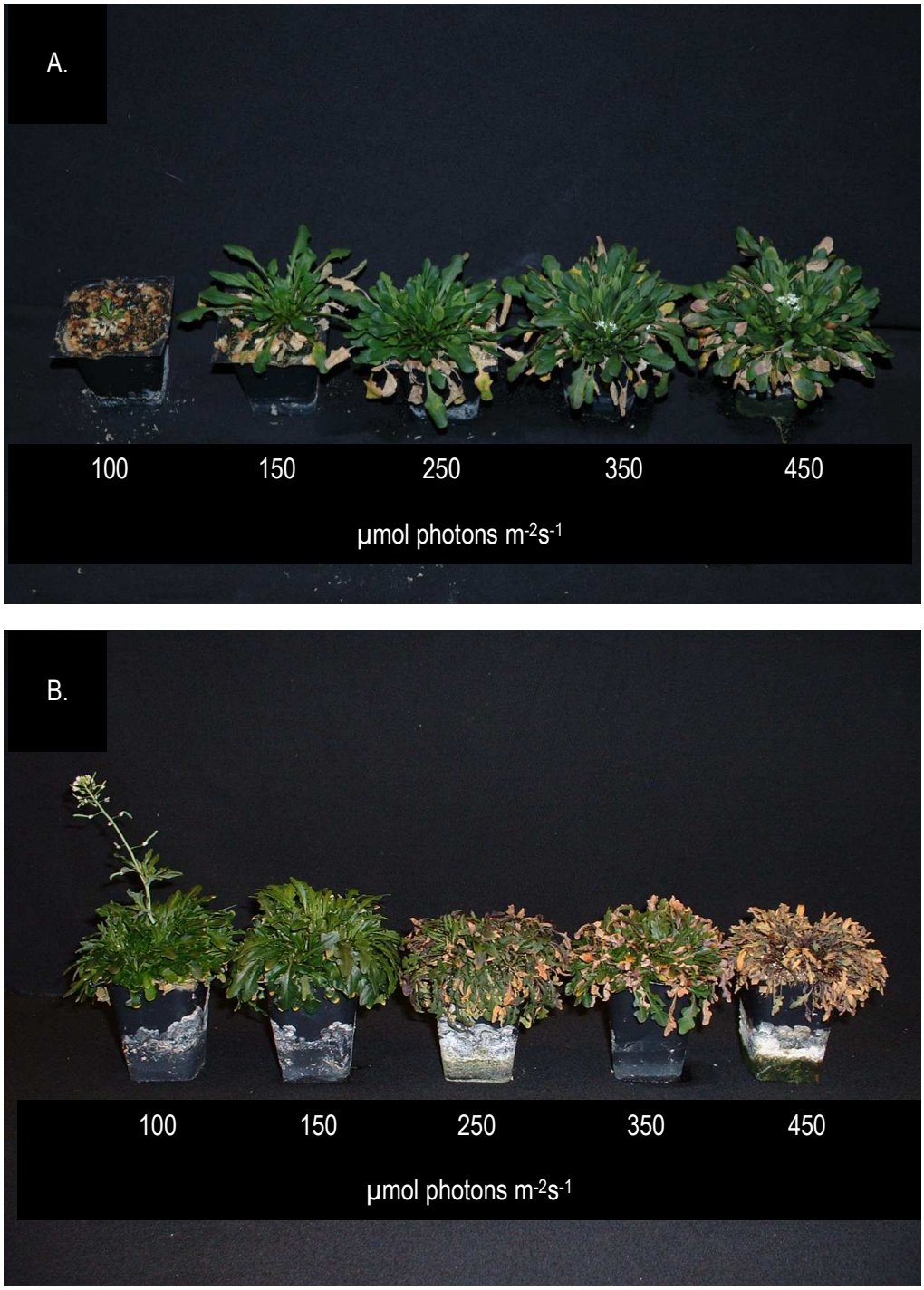


Figure 4.1. Irradiance requirements for flowering. Flowering in 65 day old Yukon (**A.**) and 115 day old Shandong (**B.**). Growth occurred at 20°C with a 16 h photoperiod and PPFD as indicated. In both cases flowering occurred without vernalization.

about 3 months (Figures 4.1B and 4.2A). Interestingly, under cold-grown conditions (4°C constant temperature with 16 h light period), all three experimental taxa exhibited a convergence of flowering time around two months at irradiances of 250 and 350 µmol photons m^{-2} s^{-1} (Figure 4.2B). Still, *Arabidopsis* was the earliest to flower with its fastest rate of growth under all growth conditions compared to both Yukon and Shandong (Figures 4.1, 4.2, 4.3, 4.4 and 4.5). Thus, phenological development, especially the reproductive transition in *Thellungiella* ecotypes and *Arabidopsis* exhibited intricate interactions between experimental taxa and environmental factors (irradiance, photoperiod and temperature).

4.3.2 Correlations Between Growth Parameters

Interestingly, the correlations among the absolute plant growth parameters appeared to be similar between taxa within a growth regime and within taxa across growth regimes. So, a combined correlation analyses between the growth parameters of all taxa was performed and the results are presented in Table 4.4.

From the Table 4.4, it is apparent that the absolute growth parameters had a significant positive correlation among each other. Leaf area (LA) and shoot biomass (FW or DW) were very highly correlated (Table 4.4). Number of rosette leaves (NRL), RR and LT had significantly positive correlations between each other and with LA and shoot biomass (FW or DW; Table 4.4). It can be inferred that any of the absolute growth parameters pertaining to leaf size and leaf mass can represent the trend of plant growth in *Thellungiella* ecotypes and *Arabidopsis*.

There was also a high correlation between the relative growth parameters, RGR and ULR (Table 4.4). While RGR was not correlated significantly with any of the absolute growth variables, ULR held significant and moderate degree of positive correlation with those variables (Table 4.4). SLA was significantly and negatively correlated with all measured and derived growth parameters (Table 4.4).

4.3.3 Growth Comparison of Plant Taxa Within Growth Regimes

Figures 4.4 and 4.5 clearly show the apparent differences in growth trend between the experimental taxa in all three growth regimes. Plants were compared for absolute and relative growth parameters measured at 4-day intervals from 17 to 37 DAS. The plant taxa exhibited significant difference for most of the growth variables (Figure 4.5). Overall, *Arabidopsis* exhibited a faster growth trend than the *Thellungiella* ecotypes in all growth regimes. Reproductive transitions occurred in *Arabidopsis* while Yukon and Shandong remained in the vegetative stage during the entire period of measurement.

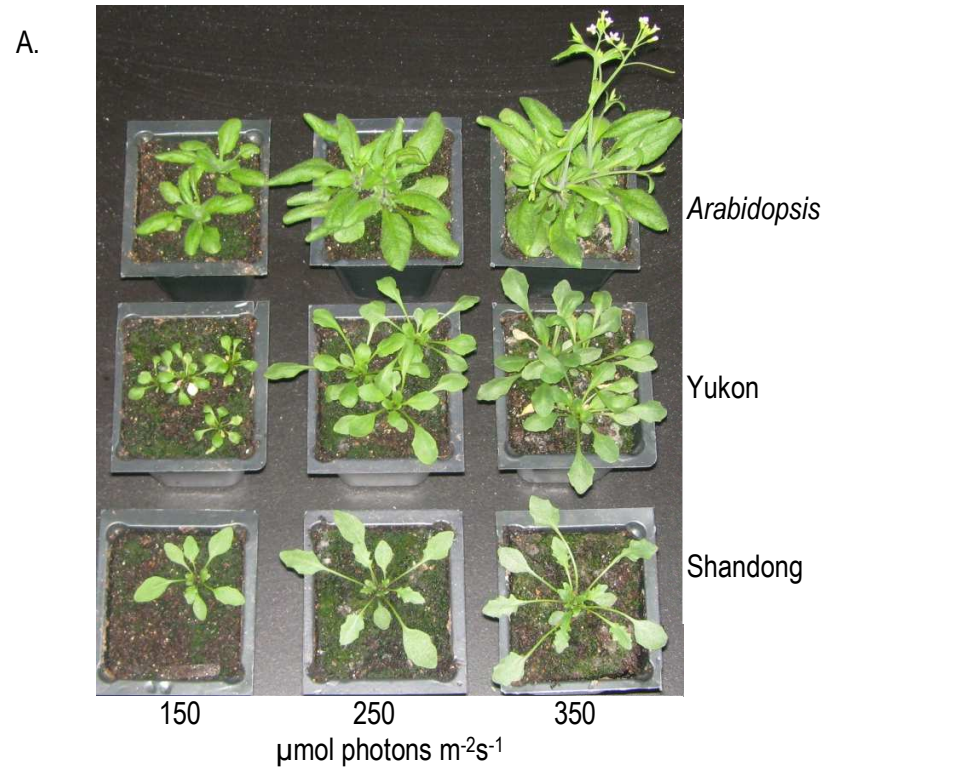


Figure 4.2. Effect of irradiance and temperature on plant phenotypes. Phenotype of the Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown at 20°C (**A.**) or 5°C (**B.**) with a 16 h photoperiod and irradiance values as indicated. The photographs were taken on the 25th day after sowing 20°C-grown and 55 days after sowing for 5°C -grown plants.

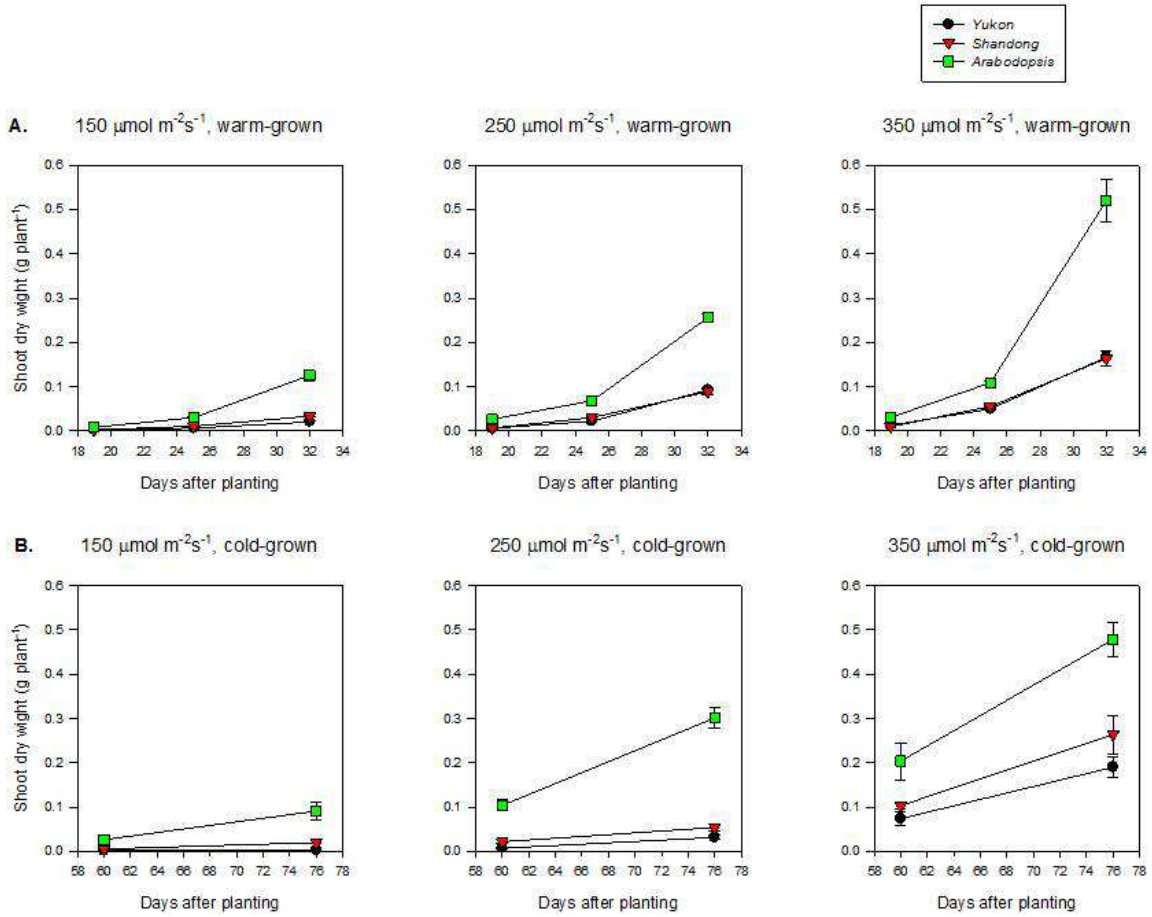


Figure 4.3. Effect of irradiance and temperature on shoot DW. DW accumulation of theYukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown at 20°C (**A.**) or 5°C (**B.**) with a 16 h photoperiod and irradiance values as indicated. Yukon ecotype (●); Shandong ecotype (▼); *Arabidopsis* (■). Values represent means ± SE (*n* = 3).

Table 4.4. Pearson correlations between growth parameters of Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis*

Growth parameter	NRL	RR	LA	FW	DW	SLA	LT	RGR
RR	0.811 (<0.001)							
LA	0.905 (<0.001)	0.793 (<0.001)						
FW	0.862 (<0.001)	0.691 (<0.001)	0.968 (<0.001)					
DW	0.881 (<0.001)	0.684 (<0.001)	0.952 (<0.001)	0.988 (<0.001)				
SLA	-0.474 (<0.001)	-0.515 (<0.001)	-0.408 (<0.001)	-0.431 (<0.001)	-0.450 (<0.001)			
LT	0.629 (<0.001)	0.605 (<0.001)	0.655 (<0.001)	0.731 (<0.001)	0.713 (<0.001)	-0.750 (<0.001)		
RGR	-0.071 (0.413)	-0.016 (0.856)	-0.024 (0.779)	0.002 (0.983)	-0.018 (0.834)	-0.035 (0.690)	0.190 (0.028)	
ULR	0.286 (0.001)	0.314 (<0.001)	0.278 (0.001)	0.337 (<0.001)	0.344 (<0.001)	-0.521 (<0.001)	0.636 (<0.001)	0.772 (<0.001)

Correlations are combined for the plant taxa compared
Values in parenthesis represent *P*-values
DW, shoot dry weight; FW, shoot fresh weight; LA, leaf area; LT, leaf thickness; NRL, number of rosette leaves; RGR, relative growth rate; RR, rosette radius; SLA, specific leaf area; ULR, unit leaf rate

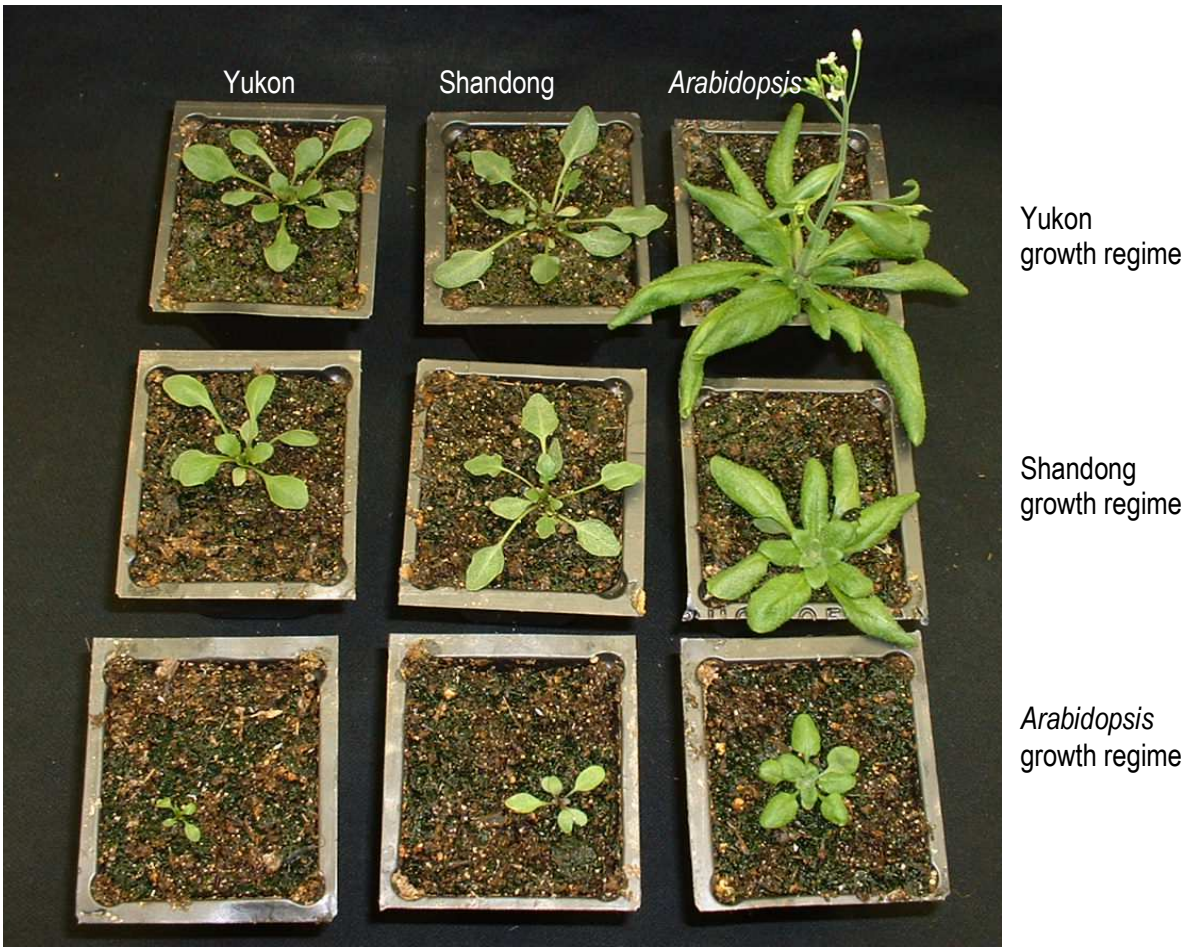


Figure 4.4. Growth phenotypes under different growth regimes. Phenotype of theYukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* **(**along the columns) were grown under Yukon, Shandong and *Arabidopsis* growth regimes (along the rows). The photographs were taken on the 25th day after sowing.

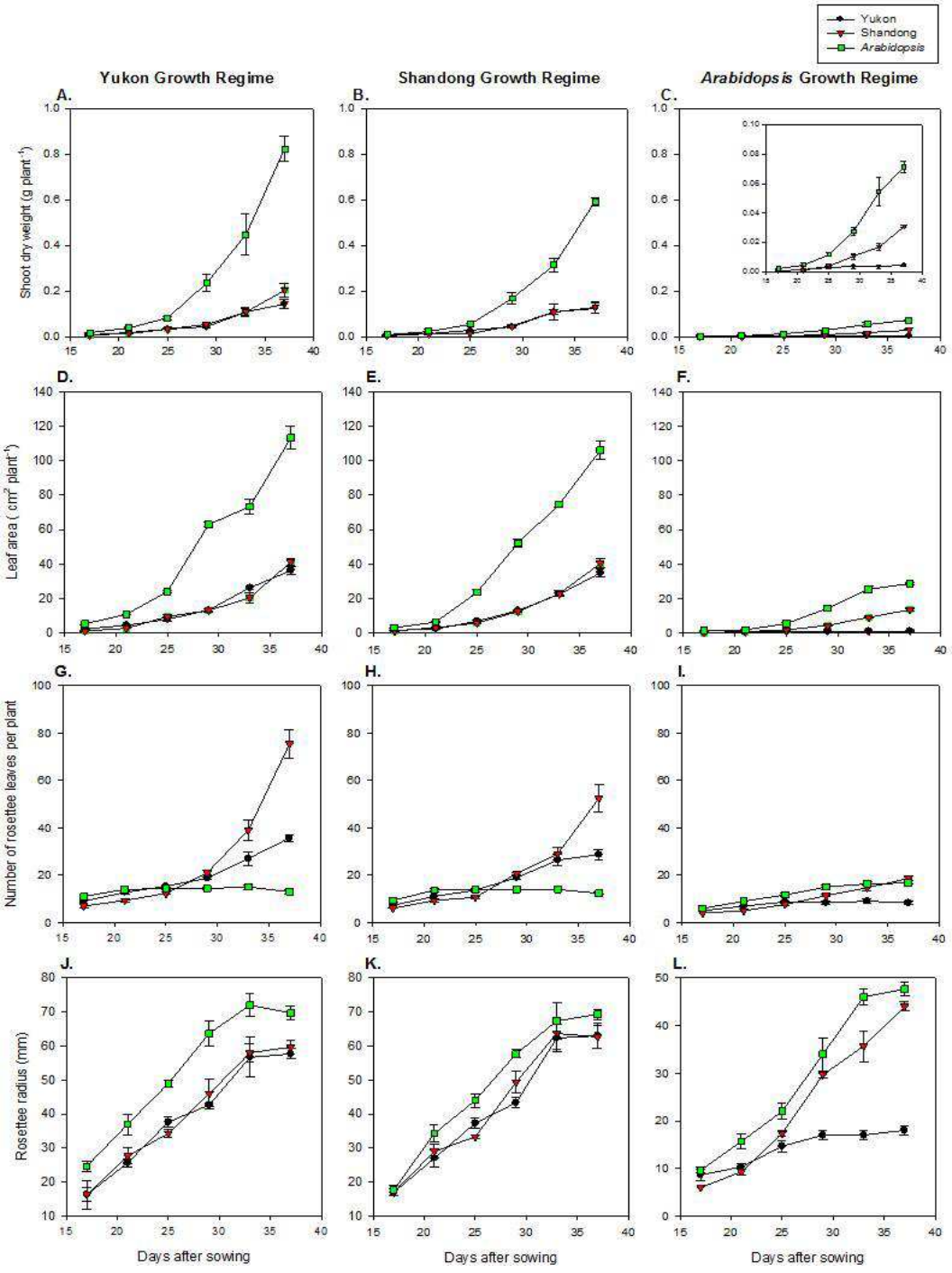


Figure 4.5. Shoot and leaf growth characteristics. DW (**A., B., C.**), LA (**D., E., F.**), NRL (**G., H., I.**), and RR (**J., K., L.**) of *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Yukon ecotype (▼); Shandong ecotype (■); *Arabidopsis* (●). Values represent means ± SE (n = 3).

4.3.3.1 Yukon Growth Regime

For the trend of shoot DW accumulation, *Arabidopsis* significantly outcompeted ($P \leq 0.0001$) Yukon and Shandong ecotypes, while the latter two remained statistically at par (Figure 4.5A). Significant differences ($P \leq 0.0001$) between the taxa were also evident for NRL (Figure 4.5G). Leaf phenology also differed significantly as reflected by highly significant taxa by plant-age interactions ($P < 0.0001$). Shandong produced significantly higher NRL followed by Yukon and then *Arabidopsis* (Figure 4.5G). However, *Arabidopsis* had significantly larger RR with higher LA ($P < 0.0001$ for both traits) than Shandong and Yukon ecotype, while the later were at par to each other for both traits (Figure 4.5D, J). Leaf development and dry matter accumulation appeared to be very similar between *Thellungiella* ecotypes. The Yukon ecotype had significantly higher SLA ($P = 0.001$) followed in order by Shandong and *Arabidopsis*. Conversely, *Arabidopsis* had significantly thicker leaves ($P < 0.0001$) followed by Shandong and Yukon ecotype (Table 4.5). This means that *Thellungiella* tends to maximize light interception with higher leaf area per unit of leaf mass. RGR (increase in dry mass per unit of plant mass over a specified period of time) was significantly higher in *Arabidopsis*, while Shandong and Yukon ecotypes were at par (Table 4.5). For ULR (growth rate per unit leaf area), no significant difference ($P = 0.22$) was observed between ecotypes (Table 4.5).

4.3.3.2 Shandong Growth Regime

The growth behaviour of plant taxa in Shandong regime largely resembled with that of Yukon regime (Figure 4.5A-B, D-E, G-H, J-K). Here also, the taxonomic differences for growth parameters were highly significant ($P \leq 0.0001$), with highly significant interactions ($P < 0.05$ to < 0.0001) between the ecotypes and plant age for the traits. As in Yukon growth regime, the trend for shoot DW accumulation was significantly higher ($P \leq 0.0001$) in *Arabidopsis* than that of the Yukon and Shandong ecotypes, with the later remaining statistically at par (Figure 4.5B). In the same token, Shandong produced significantly higher NRL ($P \leq 0.0001$) followed by Yukon and then *Arabidopsis* (Figure 4.5H). *Arabidopsis* had significantly larger RR with higher LA ($P \leq 0.0001$ for both traits) than Shandong and Yukon ecotype, while the later were at par to each other for both traits (Figure 4.5E, K). *Arabidopsis* had significantly higher ($P = 0.002$) SLA than that of Yukon, while Shandong was statistically at par with both *Arabidopsis* and Yukon (Table 4.5). *Arabidopsis* had significantly thicker leaves ($P = 0.0003$) than *Thellungiella* ecotypes and the later were at par for LT (Table 4.5). The RGR was significantly higher in *Arabidopsis* ($P = 0.001$) followed in

Table 4.5. Summary of relative growth parameters in Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown at three different regimes

Parameter	Taxa	Growth regime					
		Yukon		Shandong		*Arabidopsis*	
LT (µm)	Yukon	279.2 ± 9.3^{c}	B	324.8 ± 15.6^{b}	A	244.5 ± 9.72^{a}	C
	Shandong	349.7 ± 18.9^{b}	A	331.1 ± 16.7^{b}	A	213.5 ± 12.3^{b}	B
	Arabidopsis	424.9 ± 25.0^{a}	A	383.0 ± 26.4^{a}	B	236.6 ± 11.7^{ab}	C
SLA ($cm^2\ g^{-1}$)	Yukon	272.2 ± 11.4^{a}	B	255.4 ± 13.5^{b}	B	340.5 ± 19.8^{b}	A
	Shandong	227.3 ± 15.6^{b}	B	286.2 ± 14.7^{ab}	B	498.6 ± 26.6^{a}	A
	Arabidopsis	235.8 ± 12.3^{b}	C	297.2 ± 25.7^{a}	B	467.4 ± 29.9^{a}	A
RGR ($mg\ g^{-1}\ d^{-1}$)	Yukon	127.3 ± 35.5^{b}	A	132.2 ± 23.7^{b}	A	64.7 ± 45.8^{b}	B
	Shandong	149.9 ± 17.0^{b}	A	142.2 ± 16.6^{b}	A	171.0 ± 26.2^{a}	A
	Arabidopsis	172.7 ± 21.6^{ab}	AB	187.2 ± 21.6^{a}	A	157.8 ± 11.5^{a}	B
ULR ($mg\ cm^{-2}\ d^{-1}$)	Yukon	0.566 ± 0.126	A	0.623 ± 0.140	A	0.190 ± 0.051^{b}	B
	Shandong	0.817 ± 0.110	A	0.724 ± 0.165	AB	0.379 ± 0.083^{a}	B
	Arabidopsis	0.826 ± 0.068	A	0.712 ± 0.052	A	0.386 ± 0.062^{a}	B

Values represent grand means ± SE derived from the individual means of 5 time points of 4-day intervals from 17 to 37 DAS; *n* = 3 for each time point

The data were analyzed using repeated measurement ANOVA and the means were separated using DMRT

Values followed by small case letters along columns are significant within growth regime and the capital letters along the rows denote the significant difference of an ecotype across three growth regimes

ANOVA, anaysis of variance; DAS, days after sowing; DMRT, Duncan's multiple range test; LSD, least significant difference; LT, leaf thickness; RGR, relative growth rate; SE, standard error; SLA, specific leaf area; ULR, unit leaf rate

order by Shandong and Yukon ecotypes (Table 4.5). There was no significant difference (P = 0.81) between the ecotypes for ULR in Shandong regime (Table 4.5).

4.3.3.3 *Arabidopsis* Growth Regime

In *Arabidopsis* regime**,** *Arabidopsis* stood significantly superior to *Thellungiella* ecotypes in shoot dry matter accumulation (Figure 4.5C). Shandong also had significantly higher rate of dry matter accumulation than that of Yukon (Figure 4.5C). The differences between the taxa for NRL, RR and LA were highly significant ($P \leq 0.0001$) (Figure 4.5F, I, L) and so were the interactions between taxa and plant age ($P < 0.0001$). *Arabidopsis* had significantly rapid leaf development followed in order by Shandong and Yukon ecotype (Figure 4.5C, F, I, L). Shoot DW, SLA, RGR and ULR also differered significantly ($P < 0.01$) with significant interactions between the plant taxa and age of the plants. *Arabidopsis* and Shandong remained at par and significantly higher than the Yukon ecotype for SLA ($P < 0.0001$), RGR ($P < 0.0001$) and ULR (P = 0.01) (Table 4.5). For LT, Yukon and *Arabidopsis* stood at par and differed significantly (P = 0.05) from Shandong (Table 4.5).

4.3.4 Growth Comparison of Plant Taxa Across Growth Regimes

The growth rate of the taxa, as reflected by their trend of leaf development and increase in shoot dry matter differed significantly across growth environments (Figure 4.5). In all experimental taxa, plant growth and phenological development were faster in the Yukon growth regime followed by the Shandong growth regime, while the *Arabidopsis* regime had much slower growth (Table 4.2; Figure 4.5). Leaves of all taxa in Yukon and Shandong growth regimes were thicker with more serrated leaf margins and more purplish pigmentation on the abaxial leaf surface (Table 4.5; Figure 4.4). Conversely, those in *Arabidopsis* regime were thinner with smother leaf margins and had no apparent purplish pigmentation on the abaxial leaf surface (Table 4.5; Figure 4.4). These observations were further supported by the differences in estimated LT amd SLA (Table 4.5). *Arabidopsis* and Shandong leaves stayed green for more than four weeks of leaf age, while Yukon displayed the tendency of quicker leaf turnover. These patterns were reflected with a steady increase in dry matter accumulation in *Arabidopsis* and Shandong, and an irregular fashion of dry matter increase in Yukon (Figure 4.5A-C).

4.3.4.1 Yukon Ecotype

There were significant differences ($P < 0.01$) in growth responses of Yukon ecotype across growth environments (Figures 4.4 and 4.5). In all growth environments, Yukon showed a tendency for faster leaf turnover. For most of the growth traits, growth regimes and age of the plants also had significant interactions. In the *Arabidopsis* regime, growth of the Yukon ecotype was severely arrested after two weeks of sowing (Figure 4.5). The Yukon ecotype produced a significantly higher NRL and had significantly more LA ($P < 0.0001$ for both traits) in the Yukon growth regime followed in order by those grown in Shandong and *Arabidopsis* regime in the similar aged plants (Figure 4.5D-I). Yukon grown in *Arabidopsis* regime had a significantly smaller ($P < 0.0001$) RR than that in the other two growth regimes (Figure 4.5J-L). No significant difference was observed in RR between Yukon and Shandong regime (Figure 4.5J-L). Shoot DW of the Yukon ecotype was at par in the Yukon and Shandong regimes, and both regimes were significantly superior ($P \leq 0.0001$) to the *Arabidopsis* regime for shoot DW (Figure 4.5A-C). However, LT of the Yukon ecotype was significantly ($P < 0.0001$) higher in the Shandong regime followed by the Yukon and *Arabidopsis* regimes (Table 4.5).

Significant differences were found in the RGR of the Yukon ecotype across growth regimes (Table 4.5). Yukon plants in the *Arabidopsis* growth regime had significantly higher ($P \leq 0.0001$) SLA than in the other two regimes. The Yukon and Shandong regimes remained statistically at par for SLA (Table 4.5). No significant difference was observed for RGR of Yukon plants in the Yukon and Shandong regimes, but in the *Arabidopsis* regime, RGR was significantly lower ($P = 0.001$) (Table 4.5). Yukon plants had a significantly higher ($P = 0.03$) ULR in the Shandong and Yukon regimes compared to *Arabidopsis* regime at various age groups of the plants (Table 4.5).

4.3.4.2 Shandong Ecotype

The Shandong ecotype exhibited variation in phenology and growth trends across growth regimes (Table 4.2; Figures 4.4 and 4.5) as reflected by highly significant interactions ($P < 0.01$) between plant age and growth regime for these traits. Shandong grown in the Yukon growth regime had significantly higher NRL and larger RR ($P < 0.0001$ for both traits) followed in order by those in Shandong and *Arabidopsis* growth regimes at various ages of the plants (Figure 4.5G-L). Number of rosette leaves (NRL) in the Shandong ecotype was recorded at over 300 leaves per plant in the Yukon regime when the plants were about 2 months of age (data not shown). Shandong in the *Arabidopsis* regime had significantly lower ($P < 0.0001$) LA than Yukon and Shandong regime, while the difference between the later was non-significant

(Figure 4.5D-F). Shandong had significantly higher ($P < 0.0001$) shoot DW in the Yukon regime followed in order by the Shandong and *Arabidopsis* regimes (Figure 4.5A-C).

For LT, the Yukon and Shandong regimes were at par and differed significantly ($P < 0.0001$) from *Arabidopsis* regime (Table 4.5). Shandong exhibited relatively more consistent RGR than Yukon and *Arabidopsis* across the growth regimes (Table 4.5). While Shandong had a significantly higher SLA ($P \leq 0.0001$) and lower ULR ($P = 0.05$) in the *Arabidopsis* regime than the Shandong and Yukon regimes, there was no significant difference between the growth regimes for RGR ($P = 0.33$) (Table 4.5).

4.3.4.3 *Arabidopsis* (Columbia Ecotype)

Arabidopsis consistently showed a higher rate of dry matter accumulation across all growth environments, reaching a maximum NRL within 3 weeks in the Yukon and Shandong regimes and in 4 weeks after sowing in the *Arabidopsis* regime (Figure 4.5A-C; G-I). There was significant interaction ($P < 0.01$) between growth regime and plant age in the growth traits of *Arabidopsis*. *Arabidopsis* grown in the Yukon regime had significantly faster ($P \leq 0.0001$) rosette development, while those in the Shandong and *Arabidopsis* growth regimes were statistically at par for NRL in the same aged plants (Figure 4.5G-I). The plants in the Yukon growth regime had significantly larger ($P \leq 0.0001$) LT, DW, RR and LA, followed in order by that in the Shandong and *Arabidopsis* regimes at a given age of the plants (Table 4.5; Figure 4.5).

The SLA was significantly higher in *Arabidopsis* regime ($P \leq 0.0001$) followed in order by Shandong and Yukon growth regime (Table 4.5). The RGR of *Arabidopsis* was statistically at par between the Shandong and Yukon growth regimes, but it was significantly lower ($P = 0.004$) in the *Arabidopsis* regime compared to that of the Shandong regime (Table 4.5). The *Arabidopsis* plants grown in the *Arabidopsis* regime also had significantly lower ($P = 0.004$) ULR than other two regimes (Table 4.5).

4.3.5 Growth Plasticity in Response to Growth Irradiance and Growth Temperature

Differential growth between the experimental taxa across growth regimes is explained by the differential growth plasticity to irradiance and temperature, defined here as the percentage change in DW due to a 1% change in either environmental variable. The coefficients of DPI and MGDD in the regression equation show the magnitude of change (expressed in percentage) in DW due to 1% change in the values of the respective explanatory variables (DPI or MGDD). The Yukon ecotype showed a higher growth plasticity to DPI and lower growth plasticity to MGDD than either Shandong or *Arabidopsis* (Table 4.6). The regression coefficients show that 1% increase in DPI resulted in an approximate 2.3% increase in Yukon

DW, which is drastically higher than that of Shandong (1.61%) and *Arabidopsis* (1.63%). In contrast, the percent increase in DW with the 1% increase in DAT was highest in *Arabidopsis* (5.14%) followed by Shandong (4.81%) and Yukon (3.41%) (Table 4.6).

4.4 Discussion

While *Arabidopsis* has been extensively used in physiological, genomics, proteomics and metabolomics studies, there appears to be a divergence of growth environments used that makes it difficult for a unified comparison of the results and the ability to draw conclusions between different studies (Clifton et al. 2005). A comparative analysis of the results of *Arabidopsis* accessions with common experimental procedures revealed significant differences in the phenotypes and molecular profiles between some laboratories. Those differences were attributed to the small variations in growing conditions, especially the light quality and handling of plants **(**Massonnet et al. 2010). These results show the importance of standardization of experimental protocols and control of non-experimental variables. It is also important to perform such studies as considerable ecotypic variation may exist within a species. It is prudent to characterize different ecotypes in order to choose a robust model system that will provide a wide range of information about the interaction of plants with their environments. Now, in the post-genomics era of plant integrative and systems biology, studies are pointed towards integration of knowledge and modeling of biological processes (Bevan and Walsh 2006; Koornneef and Meinke 2010) and the importance of methodological homogeneity in molecular biological research is heightening. Detailed growth characterization and use of multiple indicators of growth and development may overcome non-experimental sources of variation while sampling plants. A survey of recent studies on *Thellungiella* and *Arabidopsis* shows the variation in the growth conditions such as temperature, irradiance and photoperiod (Appendix C). In an effort to characterize the light and temperature response of *Thellungiella* and *Arabidopsis*, growth experiments were conducted in three reference growth regimes. These growth regimes were selected based on what appeared to be representative for *Thellungiella* Yukon, *Thellungiella* Shandong and *Arabidopsis* based on the literature.

Table 4.6. Growth plasticity in Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* in response to growth irradiance and growth temperature

Taxa	Regression equation	Model diagnostics
Arabidopsis	*Ln*DW = - 31.2 + 1.63 *Ln*DPI + 5.14 *Ln* MGDD	$P < 0.001$; VIF = 1.005; R^2 = 98.7%
Shandong	*Ln*DW = - 30.3 + 1.61 *Ln*DPI + 4.81 *Ln* MGDD	$P < 0.001$; VIF = 1.001; R^2 = 96.8%
Yukon	*Ln*DW = - 23.8 + 2.33 *Ln*DPI + 3.43 *Ln* MGDD	$P < 0.001$; VIF = 1.005; R^2 = 94.9%

MGDD, modified growing degree days; DPI, daily photon irradiance; DW, shoot dry weight (in milligrams)

4.4.1 Environmental Interactions and Vernalization in *Thellungiella*

Vernalization is defined as the acquisition of the competence to flower by exposure to the prolonged cold temperature. Both Yukon and Shandong ecotypes flowered in 2-3 months under specific growth conditions without vernalization (Figure 4.1). It seems that Yukon and Shandong respond differentially to the floral induction stimuli emanated from the complex interplay of irradiance, photoperiod and temperature. Yukon grown at 350 to 450 µmol photons m^{-2} s^{-1} PPFD, 20°C daily temperature and 16 h photoperiod flowered in about 2 months (Figures 4.1A and 4.2A), while Shandong grown at 100 µmol $m^{-2}s^{-1}$ with the same temperature and photoperiod flowered in about 3 months (Figures 4.1B and 4.2A). Upon cold treatment at 4°C for 15 days (between 35 and 50 days after sowing), the *Thellungiella* plants grown in typical *Arabidopsis*, Yukon and Shandong growth regimes flowered after two months (Table 4.3). It was found that if grown under cold temperatures (4-5°C) with a moderate level of irradiance (250 to 350 µmol photons m^{-2} s^{-1}), *Thellungiella* started flowering around two months after sowing (Figure 4.2B). This observation contrasts with some earlier studies where *Thellungiella* was reported to have an obligate vernalization requirement of about 3 weeks in order to flower (Bressan et al. 2001), while without vernalization, flowering occurred only after 10 to 12 months of plant emergence (Inan et al. 2004). The contrasting observations between present and earlier studies suggest that our understanding of the growth and reproductive behavior of *Thellungiella* is still at infancy. It can be inferred from our results that appropriate light and temperature regimes can replace the vernalization requirement for *Thellungiella* ecotypes.

In this study, *Arabidopsis* showed signs of the reproductive transition at about 10th leaf stage under both warm and cold-grown conditions. This result corroborates the findings of earlier studies on *Arabidopsis* under long-day conditions (Koornneef et al. 1991; Cookson et al. 2007). However, the decrease in day length from 16 h to 12 h caused an increase in the NRL at flowering (Cookson et al. 2007). On the other hand, *Thellungiella* displayed variable responses across different growth regimes (discussed above). In the warm-grown condition where flowering occurred in *Thellungiella*, the event was preceded by the production of profuse NRL (over 60 leaves). Under the cold-grown condition, on the other hand, *Thellungiella* underwent the reproductive transition with about 10 to 15 rosette leaves per plant.

4.4.2 Phenological Development is Conserved Across Growth Environments

Thellungiella ecotypes showed slower phenological development than *Arabidopsis* across wide range of growth regimes characterized by different combinations of temperature and irradiance. However,

the ecotypic differences in growth rate and phenology significantly narrowed in continuously cold grown conditions (Figures 4.2 and 4.3). It was found that both *Thellungiella* and *Arabidopsis* can germinate and successfully complete their life cycle under a cold temperature as low as 4°C (data not shown). Our results corroborate the earlier finding by Griffith et al. (2007) who showed that *Thellungiella* Yukon germinated and reproduced under cold temperatures of 4°C. Vigil et al. (1997) and other earlier findings cited therein showed that Canola, a cultivated relative of *Arabidopsis* successfully germinates below 5°C and the growing degree hours requirement for 50% emergence was between 1500 to 2800. Out of 309 *Arabidopsis* accessions studied for their germination capacity in the cold, about 4% accessions displayed over 20% germination at 6°C in the dark. Although the study precludes the roles of light to trigger phytochrome responses during seed germination, it substantiates that *Arabidopsis* does germinate at cold temperature (Meng et al. 2008). *Arabidopsis* leaf initiation and expansion rates were found to be linearly related to the temperatures in the range of 6-26°C, with the common x-intercept of 3°C. These results suggest that 3°C is the threshold temperature for the initiation and development of *Arabidopsis* leaves (Granier et al. 2002).

4.4.3 Surrogate Measures of Shoot Biomass

Our results clearly show that any of the absolute growth variables relating to leaf size and shoot biomass can reasonably reflect plant growth of *Thellungiella* and *Arabidopsis* for vegetative plants (Table 4.4). The NRL, RR and LA can be the surrogate of shoot dry mass. This relationship may help enhance the sampling efficiency and replace the need of destructive sampling for determining the relative size of plants for phenotypic studies. There was very high degree of positive correlation between shoot FW and DW of *Thellungiella* ecotypes and *Arabidopsis*. The correlation between the LA and shoot mass (FW or DW) was also nearly perfect. Moreover, NRL, RR and LT were also significantly correlated between each other and with LA, FW and DW. These findings corroborate very well with earlier studies on 24 *Arabidopsis* accessions, where nearly perfect correlations were observed between fresh and dry biomass and LA (Cross et al. 2006). It was found earlier that LA was proportional to leaf mass and inversly proportional to leaf density-thickness. These relationships were conserved in a given species (Roderick and Cochrane 2002). With an inherent relationship between LT, SLA and dry matter content, Vile et al. (2005) established that LT can be estimated by the inverse of the product of SLA and dry matter content. The early leaf growth variables such as epidermal cell size, epidermal cell number and initiation rate were correlated to one another and with final LA, suggesting that the early leaf growth variables determine the final leaf size

(Cookson et al. 2005). So, leaf biomass and size processes are under intrinsic control of plant development and are greatly influenced by growth temperature (Granier et al. 2002).

The relationships between the absolute and relative growth parameters were variable. SLA, which provides an estimate of photosynthetic area per unit biomass, was significantly and negatively correlated with all measured and derived growth parameters. The RGR that represents plant efficiency to produce new material in a specific period and ULR, which is equivalent to net assimilation rate (NAR), representing the efficiency of the foliar area to produce new material in a specific period, had significantly positive correlation between one another. While RGR was not correlated significantly with any of the absolute growth variables, ULR held significant and moderately positive correlation with those variables. In *Arabidopsis* and *Nicotiana*, higher SLA was associated with lower ULR (Tholen et al. 2004). Li et al. (1998) also found a negative correlation between RGR and SLA, but a positive correlation between RGR and ULR in *Arabidopsis* ecotypes, which exactly matches with the present results. However, in contrast to these results, a study by Gross et al. (2006) showed that RGR calculated from 4-5 week old plants was highly correlated with LA and rosette FW at the time of harvest. The contrasting results may be due to differences in the stage of plants and the number of sampling points used for RGR calculation and its comparison with FW. In these experiments, RGR was derived as an average from 6 different sampling points between 17 and 37 days after sowing. Our results have clearly shown that there were significant interactions between the plant age and the RGR value. So, it is more representative to take average of RGR values at several points at different growth stages of the plants.

The relative growth parameters reflect photosynthetic efficiencies of the plants and are used for plant growth modeling. In a previous study, RGR was positively correlated with daily integrals of photosynthesis expressed per unit leaf area, leaf mass, and plant mass (Kruger and Volin 2005). Additionally, SLA as been used to explain a proportion of growth variation in a wide array of species and environments (Kruger and Volin 2005).

4.4.4 Differential Growth Responses Across Growth Environments

The reference growth regimes designated as *Arabidopsis* growth regime, Yukon growth regime and Shandong growth regime differed in one or more environmental factors involving temperature or irradiance or photoperiod. Compared to the Yukon growth regime, the Shandong regime had a shorter photoperiod and smaller difference in day/night temperature, while the *Arabidopsis* regime differed in both irradiance and duration of photoperiod along with a constant temperature of 20°C for both day and night. It

is obvious that irradiance, temperature and photoperiod are major determinants of plant growth and development. The Yukon and Shandong ecotypes of *Thellungiella* and the Collumbia ecotype of *Arabidopsis* showed some commonality and differences in growth responses across different growth regimes.

A common growth feature of all experimental taxa is that the Yukon regime was more conducive for faster growth and ultimately a shorter life cycle. For most of the growth parameters including leaf number, leaf size, shoot biomass, RGR and ULR, the Yukon regime was either significantly superior to the Shandong and *Arabidopsis* regimes or was at par with the Shandong regime. For SLA which is negatively correlated with all other growth variables, the order of superiority reversed with the *Arabidopsis* regime being the higher than the Yukon and Shandong regimes. In all growth regimes, Yukon showed the tendency of faster leaf turnover. *Arabidopsis* consistently showed a higher rate of dry matter accumulation across all growth environments, reaching the maximum rosette leaf number within 3 weeks in the Yukon and Shandong regimes and in 4 weeks after sowing in the *Arabidopsis* regime.

Amidst these common growth responses to the environments, some differential trends were observed between the experimental taxa. Li et al. (1998) speculated that RGR may be conservative trait in ecotypes of *Arabidopsis* across different latitudes. This property of RGR held true in our study for *Arabidopsis* and the Shandong ecotype. The only exception was in the case of the Yukon ecotype under low growth irradiance (such as in the *Arabidopsis* growth regime). Conversely, the Yukon ecotype required higher irradiance for comparable RGR with that of Shandong and *Arabidopsis*. The LT in the Yukon ecotype differed from that of *Arabidopsis* and Shandong, in that the former had higher LT in the Shandong regime and the later had higher LT in the Yukon regime (Table 4.5). Another interesting response of Yukon was that its growth was completely arrested in the *Arabidopsis* regime after two weeks of sowing, when cotyledonery food reserves were already depleted. However, new leaves were continuously formed and old ones faded away as in other growth regimes. Not surprisingly, flowering occurred in *Arabidopsis* in the chronological order of Yukon, Shandong and *Arabidopsis* regimes. No flowering took place in Yukon in any of the 3 growth regimes, nor did it in Shandong grown in Yukon and Shandong regimes. Shandong flowered after 3 months in the *Arabidopsis* growth regime.

In this study, the level of irradiance and duration of photoperiod varied simultaneously in the three different growth regimes. The expression of irradiance in terms of DPI takes into account of both instantaneous irradiance and photoperiod, making it feasible to quantify the responses of plant taxa to variation in light parameters. The Yukon ecotype showed higher growth plasticity to DPI and lower growth

plasticity to MGDD than that of Shandong and *Arabidopsis* (Table 4.6). Yukon exhibited 2.3% increase in DW with a 1% increase in the DPI. The corresponding values of increase in DW for Shandong and *Arabidopsis* were 1.61% and 1.63% respectively. On the other hand, Yukon showed the least response to temperature units expressed as MGDD. The percent increase in DW with a 1% increase in MGDD was highest in *Arabidopsis,* followed by Shandong and Yukon (Table 4.6). Cookson et al. (2007) have shown that photoperiod has major effects on the leaf initiation, but it has little effect on the final LA. In our study, there is a negligible difference in MGDD and the major cause of growth differentials across the growth regime may be due to the availability of daily irradiance for photosynthesis. Since Yukon growth was stagnant at a growth irradiance of 100 µmol photons m^{-1} s^{-1}, it can be hypothesized that this ecotype has a remarkably higher light compensation point which may be related to its innate stress tolerance mechanism.

4.5 Conclusions

Despite having close taxonomic relationships and a high degree of genetic similarity, *Thellungiella* and *Arabidopsis* differed in growth and development in response to environmental variables. However, the relative order of phenological development of *Thellungiella* and *Arabidopsis* is conserved across the diverse growth environments. A high degree of correlation between plant biomass and leaf growth variables in both *Thellungiella* and *Arabidopsis* allows using alternative growth indicators for identifying synchronous plants and increasing the homogeneity of experimental approaches for integrative biological studies. Based on evidence that Yukon has distinctly higher growth plasticity to DPI and its inability to grow and complete its lifecycle at low irradiance levels, it can be hypothesized that Yukon has higher light compensation point. This is likely coupled with higher rates of respiration to supply the higher energy demand required to operate an energetically expensive, constitutive mechanism of stress tolerance. Inherent adaptation to stress-prone habitats and the high environmental specificity of *Thellungiella* make this genus an interesting complementary model to *Arabidopsis* for developmental, physiological and evolutionary studies.

5.0 PHOTOSYNTHETIC PROPERTIES OF *Thellungiella* AND *Arabidopsis*

5.1 Introduction

Freezing tolerance tests (Chapter 3) and growth analysis (Chapter 4) demonstrated that *Thellungiella* ecotypes and *Arabidopsis* possess distinctive physiological specificities amidst several similarities of responses to growth regimes. The differences in cold acclimation capacity between the experimental taxa (results presented in Chapter 3) reflected their contrasting ecophysiological background and tempted a hypothesis that Yukon, Shandong and *Arabidopsis* possess differential capacities to modulate photosynthetic responses to high irradiance and low temperature conditions.

Photosynthesis is a highly coordinated and environmentally sensitive metabolic processes (Eberhand et al. 2008). Plant populations evolved in different ecological niches have differential adaptive specificities of photosynthetic properties (Lambers et al. 2008; Bravo et al. 2009; Yamori et al. 2010). For example, an Antarctic ecotype of *Colobanthus quitensis* displayed more efficient photosynthetic performance in terms of a higher PSII operating efficiency (Φ_{PSII}), lower excitation pressure ($1-q_L$) and a higher magnitude of the fast-relaxing component of NPQ (q_E) than an Andean ecotype under the combination of high-light and low temperature (Bravo et al. 2007). *Plantago asiatica* ecotypes from higher latitudes were found to have higher photosynthetic rates than the ecotypes from lower latitudes (Ishikawa et al. 2007). Numerous evidence substantiates that geographical separation of a population leads to the development of photosynthetic strategies as dictated by the local environmental conditions.

The use of *Arabidopsis* as a model plant has unraveled diverse, finely regulated functional flexibilities and acclimation strategies of photosynthetic metabolism in response to various environmental conditions (Stitt et al. 2010). These include various forms of energy re-routing and redox-balancing mechanisms such as non-photochemical quenching (NPQ), cyclic electron transport, chlororespiration and even alternative non-phosphorylating respiratory pathways in the mitochondria amongst others (Rizhsky et al. 2003; Escobar et al. 2006; Wormuth et al. 2007;DalCorso et al. 2008; Voss et al. 2008; Bode et al. 2009; Häusler et al. 2009; Maurino and Peterhansel 2010; Pesaresi et al. 2010; Szechyńska-Hebda et al. 2010). Adaptation of *Thellungiella* in environmental extremes may be associated with the evolution of yet uncharacterized energy balancing mechanisms. Recent studies have shown *Thellungiella* to have significantly greater tolerance than *Arabidopsis* to various stresses including salinity (Bressan et al. 2001; Inan et al. 2004; Wong et al. 2005, 2006; Stepien and Johnson 2009), cold (Griffith et al. 2007) and nutritional deficiency (Kant et al. 2008). Transcriptomic analyses of the Yukon ecotype showed stress-

specific expression of several genes along with some novel genes including those ascribed to mechanisms for photosynthetic acclimation (Wong et al. 2005; Wong et al. 2006). Several proteins including those associated with photosynthetic functions were found to be up-regulated in cold acclimated Shandong (Gao et al. 2009). In Shandong ecotype, exposure to salinity condition resulted in an up-regulation of the plastid terminal oxidase (PTOX), accounting for up to 30% of the total electron transport through PSII (ETR_{PSII}), while there was no such up-regulation in *Arabidopsis* (Stepien and Johnson 2009). Under sub-lethal salinity conditions, *Arabidopsis* manifested photosynthetic perturbations characterized by stomatal closure, inhibition of CO_2 assimilation, inhibition of ETR_{PSII}, and increases in cyclic electron flow through PSI and non-photochemical quenching (NPQ) of chlorophyll fluorescence. In contrast, in *Thellungiella*, CO_2 assimilation was only slightly affected with no significant alteration in PSI parameters, but with substantial increase in ETR_{PSII} and concomitant up-regulation of PTOX (Stepien and Johnson 2009).

Despite the growing momentum of the use of *Thellungiella* for stress tolerance studies, comparative characterization of photosynthetic properties between *Thellungiella* and *Arabidopsis* is still lacking. In view of this knowledge gap, this study attempted to characterize basic photosynthetic properties of *Thellungiella* in comparison with *Arabidopsis* under various growth regimes. As stated above, the underlying hypothesis of this study was that the contrasting ecological backgrounds of Yukon, Shandong and *Arabidopsis* are reflected by their differential modulation of photosynthetic responses. This chapter reports the results of comparative experiments aimed to characterize basic photosynthetic properties of Yukon, Shandong and *Arabidopsis* under non-acclimating and cold acclimated conditions. A property that both *Thellungiella* ecotypes shared in common while contrasting with *Arabidopsis* was that *Thellungiella* had significantly higher activity of PSI than that of *Arabidopsis* under various growth regimes. The experimental results showed that the photosynthetic correlates of *Arabidopsis* and *Thellungiella* have differential plasticity to growth irradiance and temperature conditions. Indeed, the photosynthetic correlates differed intra- and inter-specifically between the two ecotypes of *Thellungiella* and *Arabidopsis*.

5.2 Materials and Methods

5.2.1 Plant Material and Growth Conditions

Seeds of the Yukon and Shandong ecotypes of *Thellungiella salsuginea* (Pall.) O.E. Schulz as well as *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia, Col-0) were germinated and maintained as described in Section 3.2.1 using the non-acclimating growth conditions described in Table 4.1. These are referred to as Yukon, Shandong and *Arabidopsis* growth regimes.

Separate flats of plants maintained under these conditions were shifted to 5/4°C (day/night) temperatures for the Yukon and Shandong regimes and 4°C for the *Arabidopsis* regime for cold acclimation. The irradiance and photoperiod remained the same as for the non-acclimated plants in all growth regimes. Since *Arabidopsis* germinated and grew faster than Shandong and Yukon, the growth stage during transfer were matched by transferring 3-week old plants of *Arabidopsis* and 4-week old plants of Yukon and Shandong. Photosynthetic parameters of non-acclimated *Thellungiella* and *Arabidopsis* were measured after 4 weeks and 3 weeks of sowing respectively and those of cold acclimated plants were measured after 3-weeks of cold acclimation.

5.2.2 Chlorophyll a Fluorescence

5.2.2.1 Steady-State Fluorescence Quenching

Chlorophyll steady-state fluorescence quenching characteristics were determined *in vivo* using detached leaves under saturated CO_2 conditions using a XE-PAM xenon-pulse amplitude modulation fluorometer (Heinz Walz GmbH, Effeltrich, Germany) as described in detail previously (Gray et al. 2003), following the protocol of Genty et al. (1989). Measurements were performed in a Hansatech leaf-disc chamber (LD2/3; Hansatech Instruments Ltd, King's Lynn, Norfolk UK) modified with an adapter (LD/FA; Hansatech) to accept the PAM fibreoptic. The nomenclature and the derivation of the parameters were adopted from Hendrickson et al. (2004), Kramer et al. (2004), Baker et al. (2007) and Baker (2008).

Briefly, leaves were dark-adapted for 15 min prior to the onset of measurement and application of a weak measuring light (0.1 μmol photons m^{-2} s^{-1}) allowed for the determination of minimal fluorescence (F_o) in the dark-adapted state. A saturating pulse (6500 μmol photons m^{-2} s^{-1}) of light for 800 ms was used to determine the maximal fluorescence (F_m) in the dark-adapted state. The leaves were then exposed to a series of actinic PPFDs of various intensities from 55 to 1790 μmol photons m^{-2} s^{-1} until a stable, steady-state level of fluorescence (F') in the light-adapted state was achieved, approximately 10 min after switching to the next higher light level. Application of another saturating pulse to light-adapted leaf gave the maximal fluorescence in light-adapted state (F_m'). After turning off the actinic source, subsequent application of far-red (FR) light (λ_{max} 740 nm) for 4 s resulted in the determination of the minimal fluorescence (F_o') in light-adapted state. WinControl software (ver 1.93; Heinz Walz) was used in conjunction with the PAM-data acquisition system (PDA-100; Heinz Walz) to control the timing, settings and trigger signals for the various actinic and saturating pulse light sources that were established using WinControl software. Fluorescence traces were captured and analysed using the WinControl software.

From these five basic parameters obtained from the dark- and light-adapted states, other parameters described in Section 2.3.5.6 representing photochemical properties of PSII were derived as indicated in Table 5.1. Actinic PPFDs in the range of 55-1790 μmol photons m^{-2} s^{-1} were utilized for the construction of light-response curves. The partitioning of absorbed light energy into PSII photochemistry and non-photochemical processes was estimated according to the model proposed by Hedrickson et al. (2004) and adapted by other recent researchers (Guadagno et al. 2010; Losciale et al. 2011; Savitch et al. 2010). In this model, the fraction of energy utilized to drive PSII photochemistry is estimated as Φ_{PSII}, the fraction of energy dissipated as light dependent non-photochemical quenching is estimated as Φ_{NPQ} and the constitutive non-photochemical energy dissipation and fluorescence is estimated as Φ_{NO}, hence $\Phi_{PSII} + \Phi_{NPQ} + \Phi_{NO} = 1$.

Measurements were made at reciprocal temperatures so that there were four measuring conditions in each treatment; they are: warm-grown (non-acclimated) plants measured at the growth temperature (non-acclimated warm-measured, NAWM), warm-grown (non-acclimated) plants measured at low temperature (non-acclimated cold-measured, NACM), cold acclimated plants measured at the growth temperature (cold acclimated cold-measured, CACM), cold acclimated plants measured at warm (non-acclimating) temperature (cold acclimated warm-measured, CAWM). The temperature inside the cuvette was maintained by a refrigerated circulating water bath (model RC6 CS; Lauda Dr R. Wobser and Co., Lauda-Königshofen, GmbH, Germany) and matched with those of the growth temperatures for non-acclimated and cold acclimated conditions in each growth regime.

The measurement of non-acclimated and cold acclimated plants at respective growth temperatures and reciprocal temperatures allowed for the dissection of the effects of cold-shock, cold acclimation and thermal relaxation/augmentation of photosynthetic parameters by the comparison of measurements as follows:

Cold shock effect = (NAWM-NACM)/NAWM

Cold acclimative effect = (CACM-NACM)/NACM

Thermal relaxation/augmentation = (CAWM-CACM)/CACM

For the above calculations, the results of first actinic irradiance level (55 μmol photons m^{-2} s^{-1}) were excluded if not stated otherwise. The reason of exclusion is insufficient time (10 min) for the equilibration of tissue temperature to the cuvette temperature to experience the cold-shock or warming effects by the plants.

Table 5.1. Chlorophyll fluorescence parameters used throughout this thesis

Parameter	Equation
Maximum quantum efficiency of PSII photochemistry	$F_v/F_m = (F_m-F_o)/F_m$
PSII operating efficiency	$\Phi_{PSII} = (F_m'-F')/F_m' = F_q'/F_m'$ (where $F_q' = F_m'-F'$)
Excitation pressure	$1-q_L = 1- (F_q'/F_v') \times (F_o'/F')$
Non-photochemical quenching (NPQ)	$NPQ = (F_m-F_m')/F_m' = (F_m/F_m') – 1$
Efficiency of light dependent NPQ	$\Phi_{NPQ} = [(F_m-F_m')/F_m] \times (F'/F_m') = (F'/F_m')-(F'/F_m)$
Non-photochemical quenching coefficient	$q_N = 1- (F_m'-F_o')/(F_m-F_o)$
Efficiency of constitutive non-photochemical energy dissipation and fluorescence	$\Phi_{NO} = F'/F_m$
Basal fluorescence quenching coefficient	$q_O = (F_o-F_o')/F_o$
Non-cyclic electron transport rate through PSII	$ETR_{PSII} = 0.42 \times I \times \Phi_{PSII}$ [where I is the incident PPFD on the leaf and 0.42 is the product of the spectral absorbance of the leaf (84%) and the fraction of incident photons that are absorbed by PSII (50%)]

5.2.2.2 Chlorophyll Fluorescence Imaging

Chlorophyll fluorescence imaging was used to monitor photoinhibition and recovery in whole plants. Imaging was performed as described in Section 3.2.2.3 except that only numerical data was acquired. Plants were dark-adapted for 15 min prior to measurement.

5.2.3 Photoinhibitory Treatments

For the photoinhibitory treatments, Yukon, Shandong and *Arabidopsis* plants from non-acclimating conditions and their respective cold acclimated counterparts were exposed to 1700 μmol photons m^{-2} s^{-1} at 7°C for 4 h using high pressure sodium bulbs (Sylvania Lumalux LU400/Eco, Osram Sylvania Products Inc., Manchester, USA) contained within a cold room set to 2°C. The leaf temperature during photoinhibition treatment was monitored with a digital thermometer (TruTemp 3519N, Taylor, USA) as well as normal mercury thermometer (Canlab, T2025-3G, UK). The plants were allowed to recover at room light (30 μmol photons m^{-2} s^{-1}) at 22 to 24°C for 24 h. Whole plants were used for chlorophyll fluorescence imaging of F_v/F_m in cold acclimated and non-acclimated plants before and after photoinhibitory treatment, and then after 24 h of recovery. The differences in F_v/F_m values between the control plants (prior to photoinhibitory treatment) and after the photoinhibitory treatment represented the magnitude of photoinhibition of PSII. Similarly, the difference in F_v/F_m values after the photoinhibitory treatment and after releasing photoinhibitory stress represented the recovery of maximum quantum efficiency of PSII photochemistry (F_v/F_m).

5.2.4 Photosystem I Spectroscopy

The relative redox state of P_{700} was estimated *in vivo* as the light induced change in absorbance at 820 nm ($\Delta A_{820}/A_{820}$) under ambient CO_2 conditions using a PAM-101 modulated fluorometer (Walz, Effeltrich, Germany) as described in detail previously by Gray et al. (1998), following the protocol of Asada et al. (1992). For each treatment, measurements were done at reciprocal temperatures representing those of the growth temperatures for non-acclimated and cold acclimated conditions in each growth regime. The temperature was maintained by a refrigerated circulating water bath (Lauda-Königshofen, GmbH, Germany).

Briefly, FR light (λ_{max} 740 nm) at an intensity of approximately 70 μmol photons m^{-2} s^{-1} was applied on the adaxial side of the leaf in a custom designed cuvette modified to accept the PAM fiber optic. The redox state of P_{700} was evaluated as ΔA_{820} due to the formation of the cation radical (P_{700}^+). After the P_{700}^+

signal reached a steady-state level, single turnover (ST) saturating flashes (half peak width 14 μs) and multiple turnover (MT) saturating flashes (50 ms) were applied for the transient reduction of P_{700}^{+} in the presence of the background FR irradiance. WinControl software (ver 1.93; Heinz Walz) was used in conjunction with the PAM-data acquisition system (PDA-100; Heinz Walz) to control the timing, settings and trigger signals for the various light sources. Traces were captured using the WinControl software and data files were exported to a graphing program (MicoCal Origin version 6.0; MicroCal Software Inc., Northampton,MA, USA) for plotting, smoothing and integration of the areas under the curves representing the reduction of oxidized P_{700} due to the ST and MT turnover flashes. The apparent size of the intersystem electron donor pool to PSI (e^{-}/P_{700}) was estimated *in vivo* as a ratio of the area associated with the MT and ST flashes (MT area/ST area) as described by Asada et al. (1992).

5.2.5 Photosynthetic Pigment Determination

Plant pigments were extracted from leaves in 80% (v/v) acetone by grinding in a mortar and pestle followed by centrifugation for 5 min at 4,500 rpm. Pigments were quantified spectrophotometrically (SmartSpec Plus; Bio-Rad Laboratories, Hercules, California, USA) for chlorophyll *a*, *b* and total carotenoids according to the equations of Lichtenthaler and Wellburn (1983) and expressed on a leaf fresh weight and leaf area basis. Measurement of leaf weight and leaf area was carried out by using micro-balance and area meter (LI-COR Inc. Lincoln, Nebraska, USA).

5.2.6 Experimental Design and Data Analysis

The experiments were conducted in completely randomized design in the controlled growth chambers. The measurements were replicated three or more times. The data were analyzed by using descriptive statistics, correlation and analysis of variance (ANOVA) techniques with the aid of Microsoft Excel 2007, SigmaPlot 11, and Minitab 15 software.

5.3 Results

5.3.1 Comparative PSII Photochemistry of *Thellungiella* and *Arabidopsis*

It is noteworthy that the poor growth of Yukon under the non-acclimating, *Arabidopsis* growth regime (discussed in Chapter 4) resulted in a lack of measurably sized leaves for chlorophyll fluorescence measurements. However, upon the cold acclimation for 3 weeks, the Yukon leaves attained barely measurable growth allowing for chlorophyll fluorescence measurements.

5.3.1.1 Maximum Quantum Efficiency of PSII Photochemistry (F_v/F_m)

A comparison of the data suggests that the growth regime and acclimation status had minor but differential effects on F_v/F_m of Yukon, Shandong and *Arabidopsis* (Figure 5.1). There were no adverse effects of low measurement temperature on the F_v/F_m values of all the taxa compared. Therefore, the average values of both warm and low measurement temperatures are presented. The *Thellungiella* ecotypes displayed a more consistent trend of F_v/F_m than that of *Arabidopsis* across the growth regimes and acclimating conditions. However, inter-ecotypic differences in *Thellungiella* were evident by consistently higher F_v/F_m values of Shandong (0.81 to 0.84) than those of Yukon (0.79 to 0.82). Unlike *Thellungiella*, *Arabidopsis* underwent a consistent decrease in F_v/F_m values upon cold acclimation across all growth regimes. Under non-acclimated conditions, the F_v/F_m values of *Arabidopsis* (0.82 to 0.83) were comparable to those of Shandong, while the cold-acclimated values of *Arabidopsis* (0.77 to 0.80) remained lower than the non-acclimated control values. These observations suggest that *Thellungiella* ecotypes are able to cold acclimate without an apparent effect on F_v/F_m, while *Arabidopsis* acclimates under cold conditions with some down-regulation of F_v/F_m.

5.3.1.2 Excitation Pressure ($1-q_L$)

The parameter $1-q_L$ indicates the redox poise of the primary quinone electron acceptor (Q_A) of PSII. In all taxa under various environmental conditions, $1-q_L$ increased non-linearly with the increase in measuring PPFDs (Figure 5.2A-F). With respect to measurement at respective growth temperatures, $1-q_L$ light response curves of non-acclimated and cold acclimated plants clustered distinctly displaying higher $1-q_L$ in cold acclimated plants (Figure 5.2A-C). This indicates that the temperature-dependence of $1-q_L$ is not fully compensated through cold acclimation. On the other hand, measurement of non-acclimated and cold acclimated plants at reciprocal temperatures across the range of PPFDs engendered contrasting interactions of measuring irradiance and temperature (Figure 5.2D-F). With reference to the control (NAWM), the NACM resulted in the acceleration of $1-q_L$, while CAWM gave rise to a relaxation of $1-q_L$. This is due presumably to temperature-labile enzyme kinetics of photosynthetic reactions that respond instantaneously to temperature fluctuations. Similarly, the $1-q_L$ trend was also affected by the growth conditions. This is substantiated by the fact that plants grown in *Arabidopsis* growth regimes displayed a higher $1-q_L$ than those in the Yukon and Shandong growth regimes across the range of measuring PPFDs under different measuring temperatures and acclimation satus (Figure 5.2A-F). This implies that plants grown under higher irradiances have better capacity to regulate excitation energy than those grown under

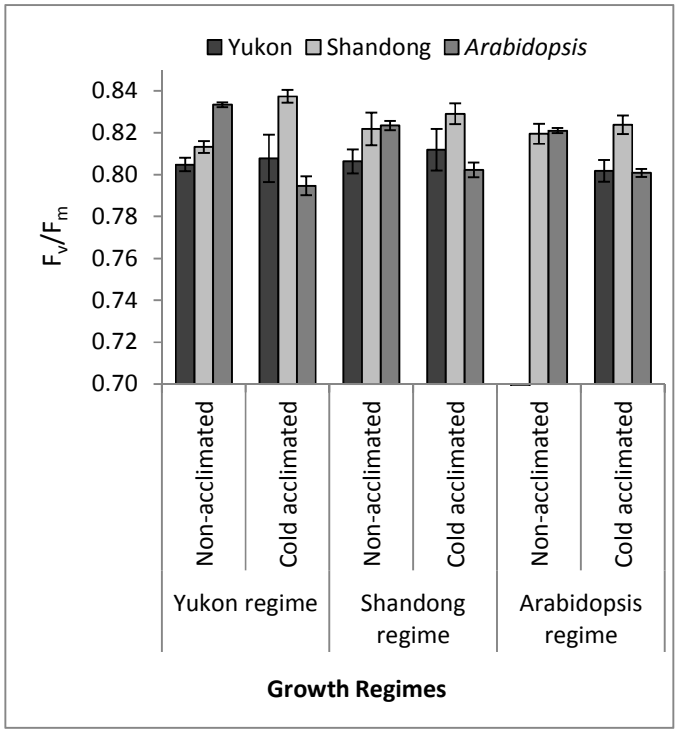


Figure 5.1. Maximum quantum efficiency (F_v/F_m) of non-acclimated and cold acclimated *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Yukon ecotype (■); Shandong ecotype (■); *Arabidopsis* (■). Values represent means ± SE (*n* = 3 to 6).

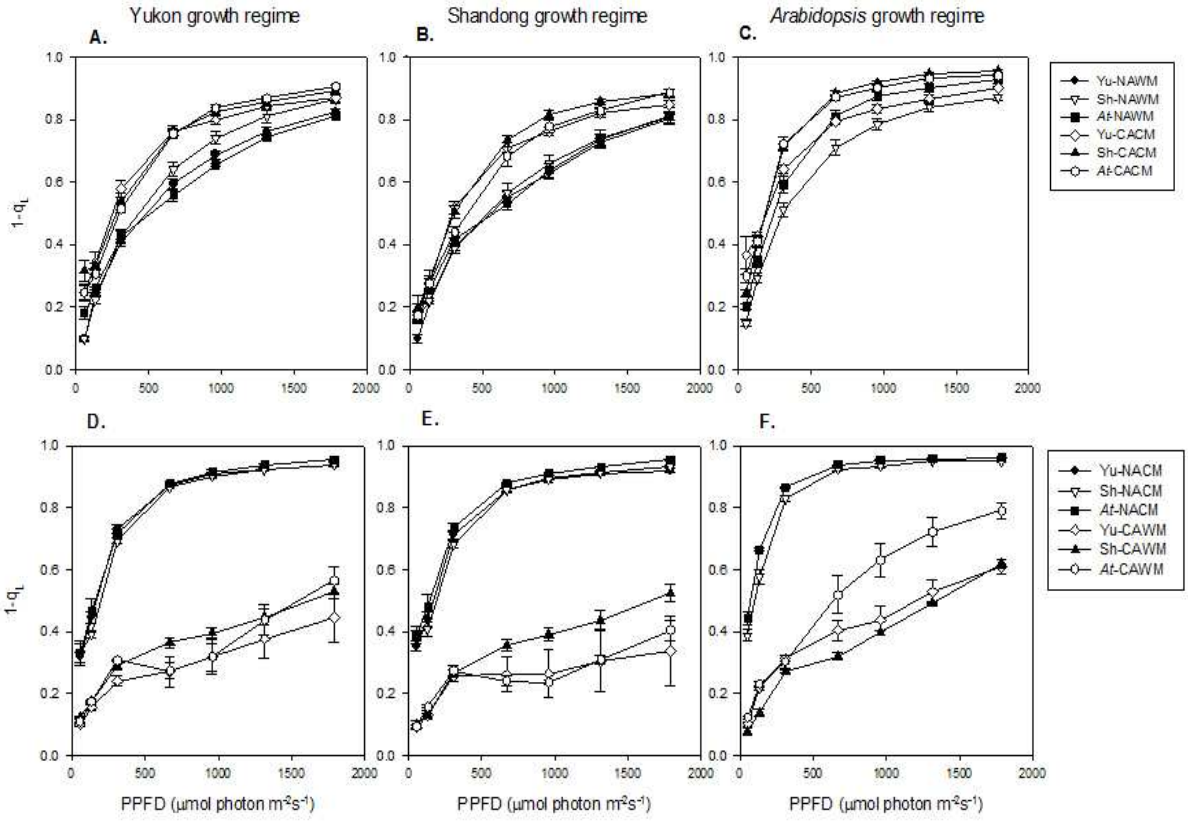


Figure 5.2. Light response curves of 1-q_L for *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Non-acclimated and cold acclimated plants of Yukon (Yu), Shandong (Sh) and *Arabidopsis* (At) measured at their respective growth temperatures (**A.-C.**) as well as reciprocal temperature measurements (**D.-F.**) are shown. Values represent means ± SE (*n* = 3 to 6). CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured.

lower irradiance. Similarly, in response to increasing PPFDs, the plants grown in Shandong growth regimes showed a slightly lower 1-q_L than those in Yukon growth regimes. Since, both Shandong and Yukon growth regimes had identical growth irradiances, the differences in 1-q_L must have arisen from the longer photoperiod in Yukon growth regime. It may be argued that longer photoperiod as the representative cue of summer conditions pre-disposes plants to a higher 1-q_L.

The taxonomic differences in 1-q_L were negligible under all experimental conditions, except for those grown in *Arabidopsis* growth regimes (Figure 5.2A-F). This regime was proved to be the sub-normal growth condition for Yukon (discussed in Chapter 4) and no plants of this ecotype in non-acclimating conditions grew to the measurable size for chlorophyll fluorescence analysis. Exclusion of the exceptional results from the *Arabidopsis* growth regime leads to the conclusion that when grown under identical conditions, *Arabidopsis* and *Thellungiella* possess similar capacities to modulate excitation pressure.

The measurement of non-acclimated plants at low temperature (cold-shock) triggered an acceleration of 1-q_L, measured as the average differences between the NAWM and NACM values (as the fraction of respective NAWM). The cold-shock effect was substantially lower in Shandong (42%) than that of *Arabidopsis* and Yukon that had identical values of 51% (Table 5.2) . The cold-shock triggered an acceleration of 1-q_L that may be an indicator of photo-sensitivity. Similarly, the cold acclimative relaxation in 1-qL, measured as the average differences between CACM and NACM values (as the fraction of respective NACM) was higher in *Arabidopsis* (20%) followed by Yukon (17%) and then Shandong (13%) (Table 5.2). When cold acclimated plants were exposed to warm (control) growth temperature, there was substantial relaxation in the trend of 1-q_L and this phenomenon is herein termed as thermal relaxation of 1-q_L. This property was estimated to be 58%, 48% and 52% on the average for Yukon, Shandong and *Arabidopsis* respectively (Table 5.2). These results clearly reveal that Shandong possesses a relatively more stable 1-q_L than Yukon and *Arabidopsis* under diverse growth regimes.

5.3.1.3 Electron Transport Rate (ETR_{PSII})

Yukon, Shandong and *Arabidopsis* exhibited comparable effects of measurement temperature, cold acclimation and growth regimes on the light response curves of ETR_{PSII}. Under the respective growth temperatures, cold acclimated plants showed less ETR_{PSII} in the range of experimental PPFDs along with light-saturation of ETR_{PSII} at lower PPFDs, compared to that of non-acclimated plants (Figure 5.3A-C). This is the indication that both *Thellungiella* and *Arabidopsis* acclimate under low temperatures with the down-regulation of photosynthesis. Measurement of non-acclimated plants at low-temperature displayed a further

Table 5.2. Effect of cold-shock, cold acclimation and thermal relaxation as the fraction of corresponding values of photosynthetic correlates for *Thellungiella* ecotypes and *Arabidopsis*

Parameters (relevant PPFD range)	Cold-shock effect with respect to corresponding control (NAWM-NACM)/NAWM			Cold-acclimative gain with respect to corresponding cold-shock (CACM-NACM)/NACM			Thermal augmentation due to warm-exposure of cold acclimated plants (CAWM-CACM)/CACM		
	Yukon	**Shandong**	***Arabidopsis***	**Yukon**	**Shandong**	***Arabidopsis***	**Yukon**	**Shandong**	***Arabidopsis***
$1-q_L$ (133 to 1790 µmol photons $m^{-2} s^{-1}$)	-0.51	-0.42	-0.51	-0.17	-0.13	-0.20	-0.58	-0.48	-0.52
ETR_{PSII} (133 to 1790 µmol photons $m^{-2} s^{-1}$)	0.54	0.49	0.57	0.34	0.32	0.40	1.05	1.18	0.94
q_N	-1.10[a]	-0.71[a]	-0.76[a]	0.05[b]	0.06[b]	0.04[b]	-0.31[c]	-0.17[c]	-0.35[c]
q_O (670 to 1790 µmol photons $m^{-2} s^{-1}$)	-0.39	-0.26	-0.99	0.16	0.13	0.30	-0.50	-0.52	-0.41

Values are averages from the Yukon and Shandong growth regimes. Results from the *Arabidopsis* growth regime were excluded due to incomplete data sets for the Yukon ecotype
Negative signs before the values indicate the direction of the treatment effect on the specific parameters
Magnitudes can be interpreted in the absolute terms

[a] Average of values between 133 to 310 µmol photons $m^{-2} s^{-1}$; [b] average of values between 670 to 1790 µmol photons $m^{-2} s^{-1}$; [c] average of values between 133 to 670 µmol photons $m^{-2} s^{-1}$
There were negligible treatment effects beyond the above indicated irradiance levels
CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; ETR_{PSII}, non-cyclic electron transport rate through PSII; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured; q_N, non-photochemical quenching coefficient; q_O, basal fluorescence quenching coefficient; $1-q_L$, excitation pressure

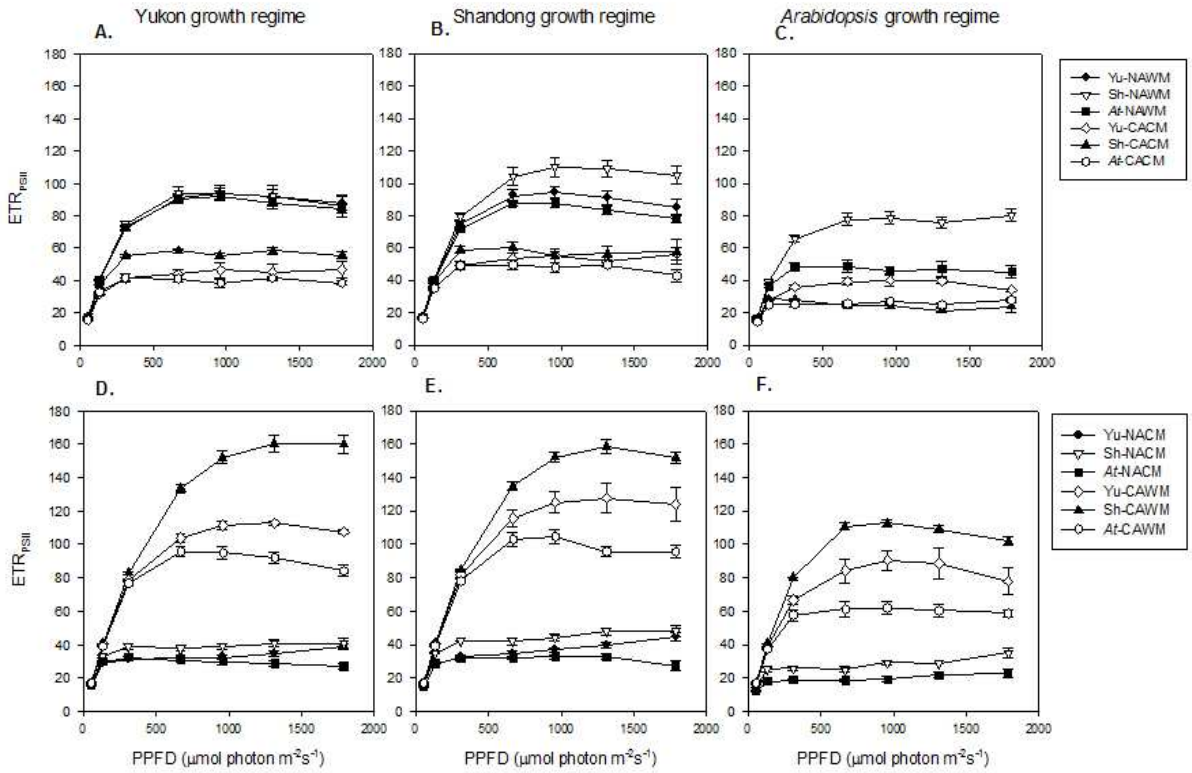


Figure 5.3. Light response curves of ETR_{PSII} for *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Non-acclimated and cold acclimated plants of Yukon (Yu), Shandong (Sh) and *Arabidopsis* (At) measured at their respective growth temperatures (**A.-C.**) as well as reciprocal temperature measurements (**D.-F.**) are shown. Values represent means ± SE (*n* = 3 to 6). CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured.

depression of ETR_{PSII} as the indication of an inhibitory effect of cold-shock on photosynthesis of non-acclimated plants (Figure 5.3D-F). On the other hand, measurement of cold acclimated plants at warm temperature augmented the ETR_{PSII} that substantially exceeded the control values of ETR_{PSII} from NAWM plants. Similarly, compared to the ETR_{PSII} of NACM, the CACM values were higher in the light response curves. This suggests that there was a gain in ETR_{PSII} due to cold acclimation from the values of the cold-shock condition.

Amidst the common trends of ETR_{PSII} displayed by the experimental taxa across various growth regimes, some quantitative differences between their responses were also evident. Shandong generally outperformed both of its counterparts in that *Arabidopsis* remained mostly at the lower scale, while Yukon lied in intermediate position between Shandong and *Arabidopsis* in the light response curves. However, there are few exceptions to this generalization indicating the differential interactions between the genotype and environmental conditions (Figure 5.3A-F).

The cold-shock triggered depression of ETR_{PSII} (measured as the average differences between the NAWM and NACM values as the fraction of respective NAWM) was highest in *Arabidopsis* and lowest in Shandong across all growth regimes (Table 5.2). The average depression, as an indication of photoinhibition, was 49% in Shandong, 55% in Yukon and 57% in *Arabidopsis*. On the other hand, the cold acclimative gain in ETR_{PSII} (measured as the average differences between CACM and NACM values as the fraction of respective NACM) were higher in *Arabidopsis* (40%) followed by Yukon (34%) and then Shandong (32%). When cold acclimated plants were exposed to warm (control) growth temperature, there was a rapid escalation of ETR_{PSII} and this phenomenon is herein termed as thermal augmentation of ETR_{PSII}. On the average, this property was estimated to be 118%, 105% and 94%, for Shandong, Yukon and *Arabidopsis* respectively (Table 5.2). These results clearly reveal that Shandong places itself in distinctive position in ETR_{PSII} properties under diverse growth regimes. Furthermore, the comparable results between the *Arabidopsis* and *Thellungiella* suggest that photosynthetic competence of *Arabidopsis* is similar to that of *Thellungiella* when grown under similar growth regimes.

5.3.1.4 Non-Photochemical Quenching (q_N)

The coefficient q_N is a measure of the thermal dissipation of absorbed excitation energy. *Thellungiella* ecotypes and *Arabidopsis* showed quantitative differences of q_N in response to increasing PPFD. The q_N trend also displayed sensitivity to low measurement temperature, acclimation status, and growth regime (Figure 5.4A-F). When measured at their respective growth temperatures, the q_N curves of

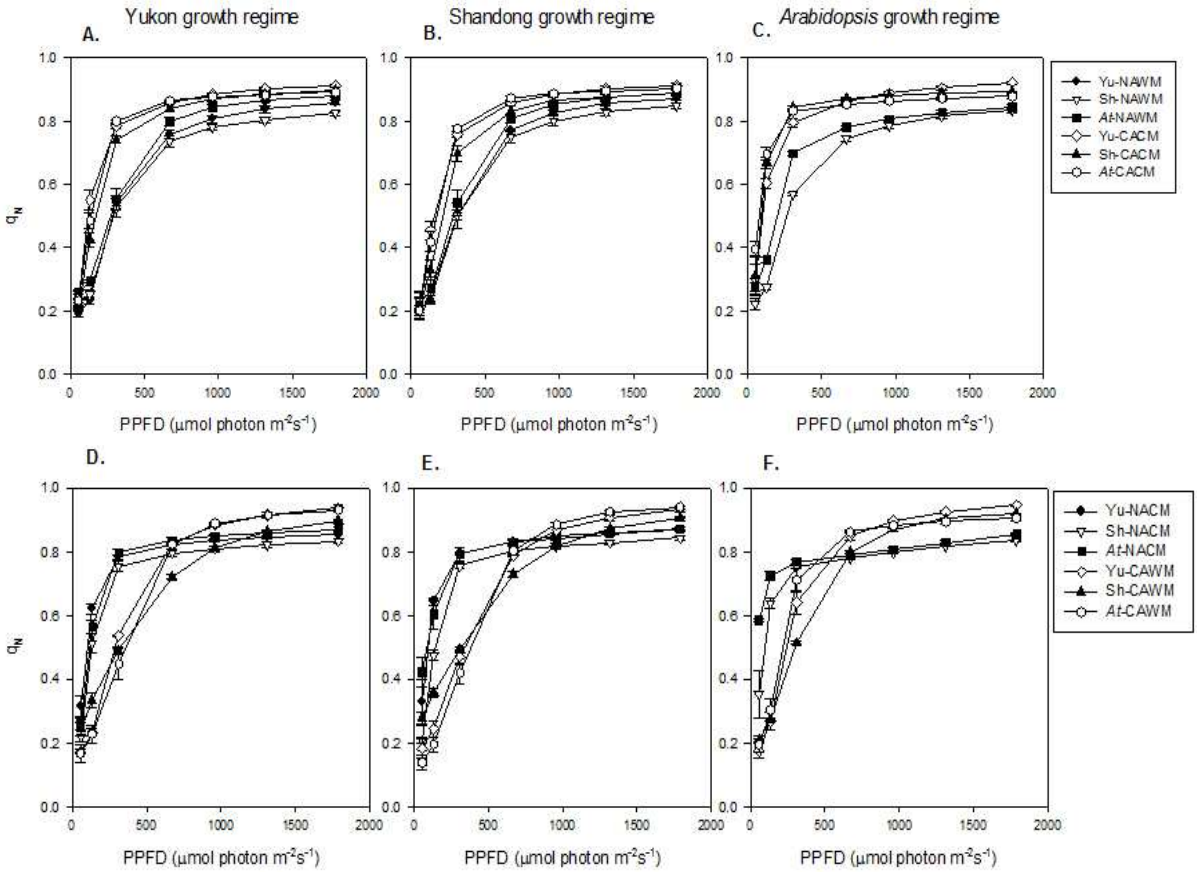


Figure 5.4. Light response curves of q_N for *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Non-acclimated and cold acclimated plants of Yukon (Yu), Shandong (Sh) and *Arabidopsis* (At) measured at their respective growth temperatures (**A.-C.**) as well as reciprocal temperature measurements (**D.-F.**) are shown. Values represent means ± SE (*n* = 3 to 6). CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured.

the cold acclimated plants (CACM) of all three taxa remained at a substantially higher level than those of their non-acclimated counterparts throughout the range of experimental PPFDs (Figure 5.4A-C). In other words, the cold acclimated plants showed a more rapid increase in q_N with the points of inflection in the light response curve remaining at lower irradiance. Contrarily, when measured at reciprocal temperatures (NACM and CAWM), the non-acclimated plants underwent a much faster increase in qN compared to cold acclimated plants (Figure 5.4D-F). At the same time, the point of inflection in light response curve of q_N remained at much lower irradiances in CAWM than those of NACM.

Amidst the above generalized trends, q_N values in the light response curves were consistently lower for Shandong and higher for *Arabidopsis*, while Yukon lied in an intermediate position (Figure 5.4A-F). These observations support the explanation as to why Shandong maintained a superior trend of ETR_{PSII} than Yukon and *Arabidopsis*.

The measurement of non-acclimated plants at low temperature (cold-shock) caused an accelerative rise in q_N with increased PPFD until 310 µmol photons m^{-2} s^{-1} and thereby stabilizing the q_N with little effect of further increase in irradiance. This may be due to the fact that enzymatic restrictions at low temperature exhausted the capacity of plants for non-photochemical quenching. The cold-shock triggered acceleration of q_N to this point of inflection (measured as the average differences between the NAWM and NACM values as the fraction of respective NAWM) was significantly higher in Yukon (110% on the average) followed in order by Shandong (71%) and *Arabidopsis* (76%) (Table 5.2). On the other hand, there was no cold acclimative gain in q_N (measured as the average differences between CACM and NACM values as the fraction of respective NACM) with the increase in PPFD until 310 µmol photons m^{-2} s^{-1}. However, a small but consistent cold acclimative gain was evident with the rise in PPFD beyond 310 µmol photons m^{-2} s^{-1}. The irradiance dependent cold acclimative gain in q_N was more or less similar (4 to 6%) for both *Thellungiella* and *Arabidopsis*. When cold acclimated plants were exposed to warm (control) growth temperatures, the q_N of all taxa raised more gradually than the control with increasing irradiances until a PPFD of 670 µmol photons m^{-2} s^{-1} and beyond which the light response of this parameter stagnated (Figure 5.4D-F). This phenomenon of warmth-triggered stabilization of q_N with the rise in PPFD is herein termed as thermal relaxation of q_N. The thermal relaxation of q_N up to the PPFD of 670 µmol photons m^{-2} s^{-1} was relatively higher in *Arabidopsis* (35%) followed closely by Yukon (31%), while Shandong remained significantly lower (17%) in this property (Table 5.2). This result again indicates that Shandong has more stable pattern of partitioning excitation energy than that of Yukon and *Arabidopsis*. This property will be further examined in the following section 5.3.1.6.

5.3.1.5 Basal Fluorescence Quenching (q_O)

The coefficient q_O is an indicator of dissipation of excitation energy from light-harvesting antenna of PSII (antenna quenching). Excluding the results of the *Arabidopsis* growth regime where a complete set of comparative data were lacking, cold acclimated plants had consistently higher values of q_O than non-acclimated plants when measured at their respective growth temperatures (Figure 5.5A-C). The results of reciprocal measurement temperatures were virtually opposite from those of growth temperatures where light response curves of non-acclimated plants remained consistently above those of cold acclimated plants (Figure 5.5A-C). All experimental taxa exhibited similar responses of q_O upon cold acclimation. On the other hand, under non-acclimated conditions the q_O trend of *Arabidopsis* remained quantitatively lower than *Thellungiella* ecotypes (Figure 5.5 A-C).

The experimental taxa exhibited differential effect of cold acclimation, cold-shock and thermal relaxation on q_O. These responses were well discernible at PPFDs higher than 310 µmol photons m^{-2} s^{-1} (Figure 5.5A-F). The cold-shock triggered a rise in q_O (measured as the average differences between the NAWM and NACM values as the fraction of NAWM) which was calculated for the irradiance levels higher than 310 µmol photons m^{-2} s^{-1}. The cold-shock triggered rise in q_O was significantly higher in *Arabidopsis* (99% on the average) followed distantly by Yukon (39%) and Shandong (26%) (Table 5.2). Similarly, there was cold-acclimative gain in q_O (measured as the average differences between CACM and NACM values as the fraction of NACM) with the increases in PPFD beyond 310 µmol photons m^{-2} s^{-1}. The cold acclimative gain in q_O was significantly higher in *Arabidopsis* (30% on the average) followed by Yukon (16%) and Shandong (13%). When cold acclimated plants were exposed to warm (control) growth temperatures there was substantial down-shift in the light response curve of q_O. Such a subsidence in q_O due to warm temperature was estimated for the PPFD levels above 310 µmol photons m^{-2} s^{-1}. This phenomenon of warmth-triggered subsidence of q_O with the rise in PPFD is herein termed as thermal relaxation of q_O. The thermal relaxation of q_O was higher in *Thellungiella* (50% in Yukon and 52% in Shandong) than that in *Arabidopsis* (41%) (Table 5.2). These results show that the cold-shock sensitivity and cold acclimative effect on q_O was more pronounced in *Arabidopsis* than in *Thellungiella*.

5.3.1.6 Excitation Energy Partitioning with Increasing Irradiances

The increase in measurement PPFD from 55 to 1790 µmol photons m^{-2} s^{-1} resulted in the non-linear decrease in the efficiency of PSII photochemistry (Φ_{PSII}) with the concomitant increase in the non-

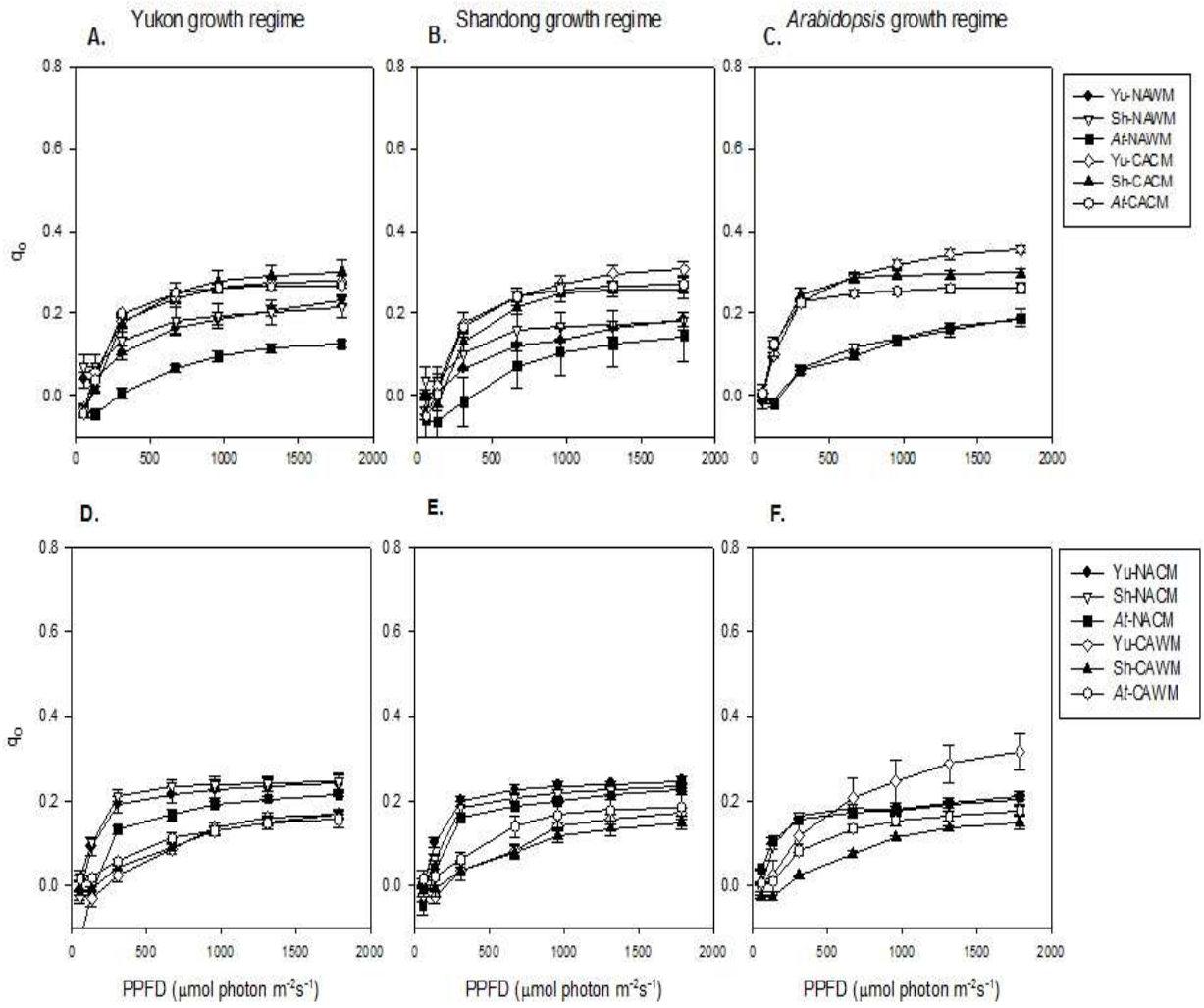
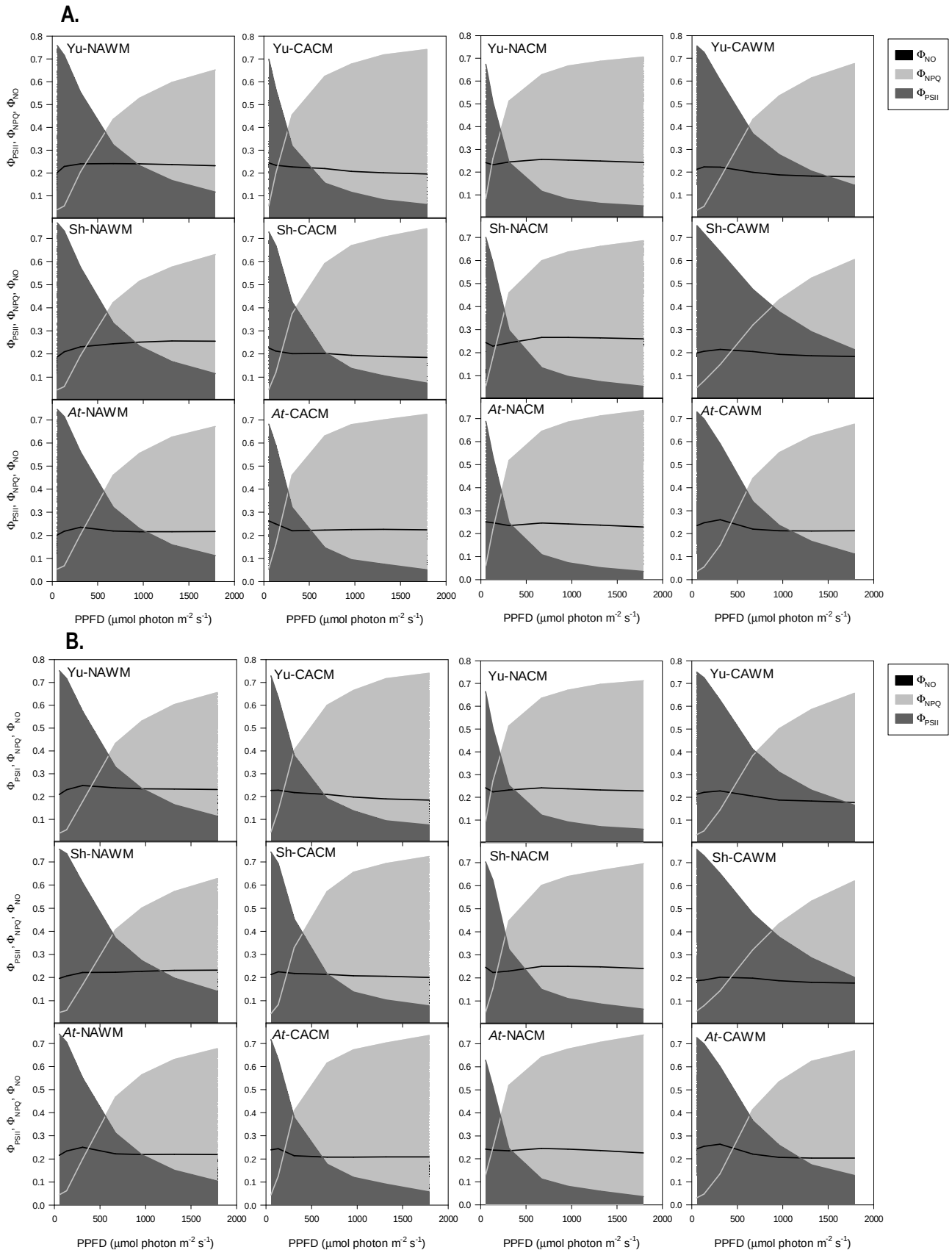


Figure 5.5. Light response curves of q_O for *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Non-acclimated and cold acclimated plants of Yukon (Yu), Shandong (Sh) and *Arabidopsis* (At) measured at their respective growth temperatures (**A.-C.**) as well as reciprocal temperature measurements (**D.-F.**) are shown. Values represent means ± SE (*n* = 3 to 6). CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured

photochemical dissipation of the excitation energy in all growth regimes and measurement temperatures (Figure 5.6A-C). The fraction of dissipated energy was discerned into two components: the first component being the light independent, constitutive non-photochemical energy dissipation and fluorescence (Φ_{NO}), and the second component being the light regulated, predominantly ΔpH-and/or xanthophyll-dependent non-photochemical dissipation within the PSII antenna (Φ_{NPQ}). The Φ_{NO} exhibited a negligible effect of experimental taxa, growth regimes and measurement temperature, and remained more or less constant around the value of 0.2. It was the Φ_{NPQ} that competed with Φ_{PSII} in the excitation energy partitioning in response to changes in the measurement temperature, measurement irradiance and the growth regimes (Figure 5.6A-C).

The acclimation status of plants and measurement temperature triggered a marked effect, while the growth regimes and plant taxa had only subtle effects on the partitioning of the excitation energy. When measured at respective growth temperatures, cold acclimated plants responded to increasing PPFD with a more rapid down-regulation of Φ_{PSII} with a proportionate increase in Φ_{NPQ} (Figure 5.6A-C). For example, the non-acclimated plants measured at growth temperature (non-acclimated warm-measured, NAWM) displayed higher Φ_{PSII} than Φ_{NPQ} until the PPFD of 300 to 630 µmol photons m^{-2} s^{-1} depending on the taxa from three different growth regimes. In contrast, the cold acclimated plants measured at low temperature (cold acclimated cold measured, CACM) showed more competitive Φ_{NPQ} that surpassed Φ_{PSII} beyond the PPFD of 155 to 380 µmol photons m^{-2} s^{-1} depending on the growth regimes and taxa (Figure 5.6A-C). Measurement at reciprocal temperatures showed a more contrasting trend of energy partitioning in non-acclimated and cold acclimated plants (Figure 5.6A-C). The Φ_{PSII} was surpassed by Φ_{NPQ} at much lower PPFDs (95 to 265 µmol photons m^{-2} s^{-1}) in non-acclimated low temperature-measured (NACM) plants compared to the cold acclimated warm-measured (CAWM) counterparts (390 to 880 µmol photons m^{-2} s^{-1}). The pattern of energy partitioning measured at 960 µmol photons m^{-2} s^{-1} at warm temperature were similar to those obtained from the measurement at 310 µmol photons m^{-2} s^{-1} at low temperature (Figure 5.6A-C). Similarly, the non-acclimated warm-measured values at 670 µmol photons m^{-2} s^{-1} were similar to those of cold-acclimated low temperature-measured values at PPFD of 310 µmol photons m^{-2} s^{-1}. It suggests that the conditions exerting higher excitation pressure (high irradiance and low temperature) lead to the energy partitioning in favour of Φ_{NPQ} at the expense of $\Phi_{PSII.}$ This aspect is further examined in the following Section 5.3.1.8. These results also suggest that cold acclimation of photosynthesis results in only partial recovery of photochemistry in *Arabidopsis* and *Thellungiella* plants. The effect of growth regimes on energy


A.
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At-CAWM
ΦNO
ΦNPQ
ΦPSII
ΦPSII, ΦNPQ, ΦNO
PPFD (µmol photon m-2 s-1)
B.
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Sh-CAWM
At-NAWM
At-CACM
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ΦNO
ΦNPQ
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PPFD (µmol photon m-2 s-1)

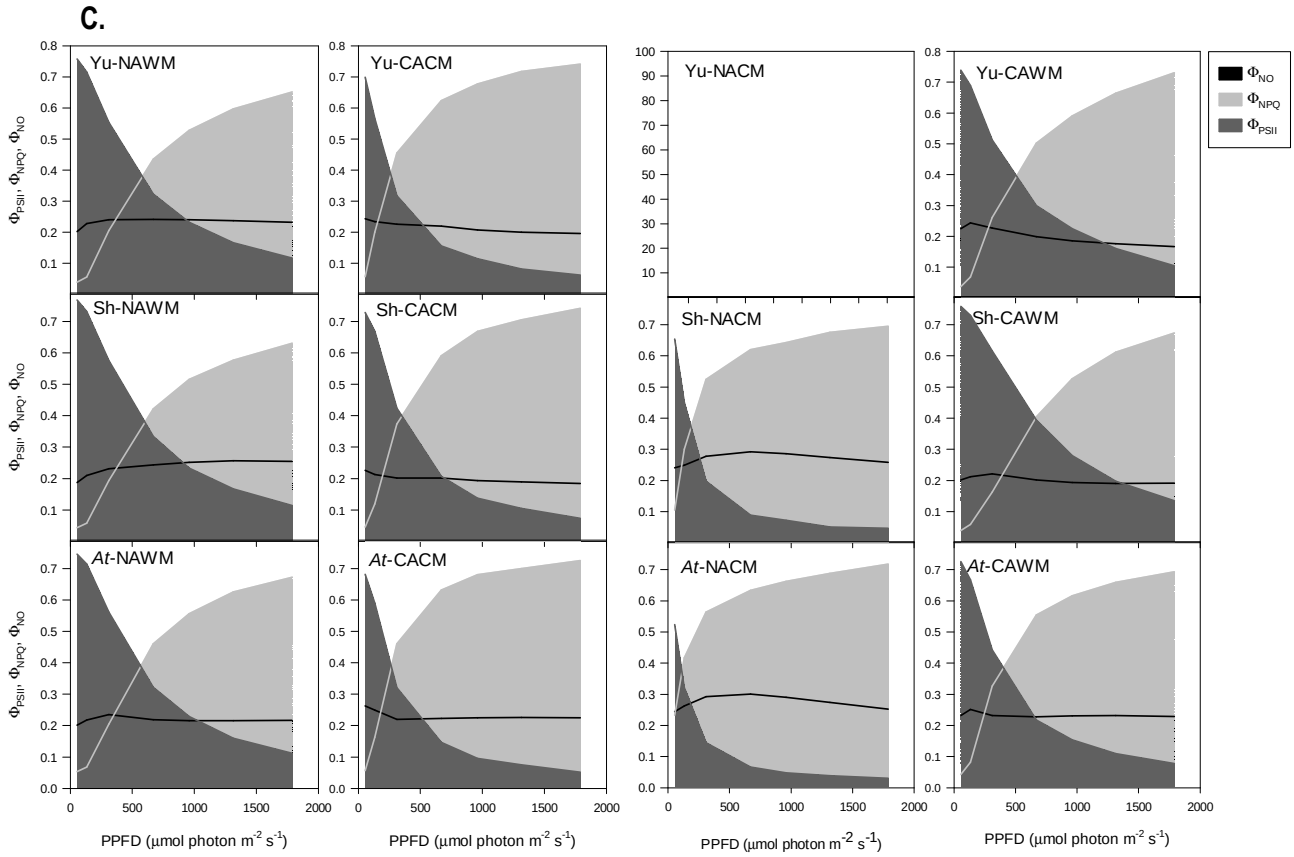


Figure 5.6. Partitioning of excitation energy as a function of irradiance for *Thellungiella* ecotypes and *Arabidopsis*. The fraction of excitation energy flow via PSII photochemistry (Φ_{PSII}) and non-photochemical dissipation pathways (Φ_{NO} and Φ_{NPQ}) in Yukon (Yu), Shandong (Sh) and *Arabidopsis* (At) was estimated for growth under Yukon (**A.**), Shandong (**B.**) and *Arabidopsis* (**C.**) growth regimes. Plants were measured at their respective growth temperatures as well as reciprocal temperature measurements as indicated. The blank graphs of Yukon ecotype in the *Arabidopsis* growth regime denotes no results obtained due to poor growth of the ecotype in that condition. CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured.

partitioning was also evident. In general, at given PPFD levels and experimental conditions the *Arabidopsis* growth regime had lower Φ_{PSII} and higher Φ_{NPQ} than the Yukon and Shandong growth regimes in the given conditions (Figure 5.6A-C). For example, in *Arabidopsis* growth regime, the range of measurement PPFDs in which the Φ_{PSII} and Φ_{NPQ} curves intersected each other were 95 to 515 µmol photons m^{-2} s^{-1}, which is much lower than the corresponding PPFDs in Shandong and Yukon growth regimes (210 to 870 µmol photons m^{-2} s^{-1}). The energy partitioning patterns at an irradiance of 960 µmol photons m^{-2} s^{-1} in the Yukon and Shandong growth regimes were comparable to that of the *Arabidopsis* growth regime at 670 µmol photons m^{-2} s^{-1}. This shows that growth conditions with higher irradiance are conducive to the development of more efficient photochemistry to utilize the intercepted irradiance.

Taxonomic differences in energy partitioning were apparent under certain circumstances. Barring few exceptions, energy partitioning in Shandong ecotype of *Thellungiella* appeared more favourable for photochemistry than that of Yukon and *Arabidopsis*, while the later appeared similar to each other across all growth regimes and measurement conditions (Figure 5.6A-C). In the Yukon growth regime, the point of intersection of competing energy pathways (Φ_{PSII} and Φ_{NPQ}) in the Shandong ecotype occurred at PPFDs of 590, 345, 250 and 880 µmol photons m^{-2} s^{-1} for NAWM, CACM, NACM and CAWM treatments respectively. The corresponding PPFDs were 560, 225, 210 and 605 µmol photons m^{-2} s^{-1} in Yukon, and those in *Arabidopsis* were 555, 255, 225 and 600 µmol photons m^{-2} s^{-1} respectively. Similarly, in the Shandong growth regime, the point of intersection of Φ_{PSII} and Φ_{NPQ} in Shandong ecotype fell around the PPFD of 630, 380, 265 and 870 µmol photons m^{-2} s^{-1} for NAWM, CACM, NACM and CAWM treatments respectively. The corresponding PPFDs in Yukon were 585, 290, 218 and 695 µmol photons m^{-2} s^{-1} and those in *Arabidopsis* were 555, 285, 210 and 620 µmol photons m^{-2} s^{-1} respectively. In the *Arabidopsis* growth regime, the comparison of energy-partitioning performance of Yukon ecotype was marred due to its sub-normal growth. Shandong displayed a consistently greater amount of energy partitioning in favour of photochemistry in the *Arabidopsis* growth regime. In this regime, the Shandong ecotype displayed points of intersection between Φ_{PSII} and Φ_{NPQ} around PPFDs of 515, 150, 180 and 645 µmol photons m^{-2} s^{-1} in NAWM, CACM, NACM and CAWM treatments respectively, compared to the corresponding PPFD of 300, 115, 95 and 390 µmol photons m^{-2} s^{-1} in *Arabidopsis*.

5.3.1.7 Inter-Relationship between Photosynthetic Correlates of PSII

It is an intriguing to examine whether the key photosynthetic correlates of *Thellungiella* ecotypes and *Arabidopsis* respond similarly to excitation pressure. Separate regression results of Shandong, Yukon

and *Arabidopsis* display that excitation pressure fairly explains the variation in photochemical and non-photochemical correlates of photosynthesis (Figure 5.7). Though the trends of all experimental taxa were also fairly similar, some differences in slopes were distinguishable. In general, it appears that Shandong is quantitatively more responsive to excitation pressure for Φ_{PSII} and Φ_{NPQ} , Yukon for q_O and *Arabidopsis* for q_N (Figure 5.7).

5.3.1.8 Excitation Energy Partitioning with Increasing Excitation Pressure

The high correlation of excitation pressure with photochemical and non-photochemical quenching parameters led to the consideration that excitation pressure may serve as the unifying determinant of energy partitioning. To examine the pattern of energy partitioning in response to excitation pressure, the results of energy fractionation from different growth regimes and measurement conditions were plotted against the excitation pressure (Figure 5.8A-C).

Differential patterns of energy partitioning at the same level of excitation pressure under different experimental conditions implies that genetic and various environmental factors (growth regimes, acclimation status, measurement temperature and measurement irradiance) have some specific effects on energy partitioning that cannot be ascribed for the short-term changes in excitation pressure. However, barring the case of CAWM conditions where the excitation pressure was greatly relaxed, it was found that excitation pressure of around 0.5 is critical for relative predominance of Φ_{PSII} or Φ_{NPQ}. When measured at growth irradiances, the intersection of Φ_{PSII} or Φ_{NPQ} curves occurred between the excitation pressure of 0.49 and 0.62 in non-acclimated plants and between 0.42 to 0.56 in cold acclimated plants (Figure 5.8A-C). *Arabidopsis* displayed relatively lower Φ_{PSII} or higher Φ_{NPQ} at given excitation pressure than *Thellungiella* ecotypes. At the reciprocal measurement temperatures, cold acclimated plants displayed higher sensitivity of energy partitioning pattern to the changes in excitation pressure than the non-acclimated plants (Figure 5.8A-C). This suggests that cold acclimation results in the development of more responsive mechanisms that detect changes in environmental conditions, thereby enabling them to adjust the pattern of excitation energy partitioning.

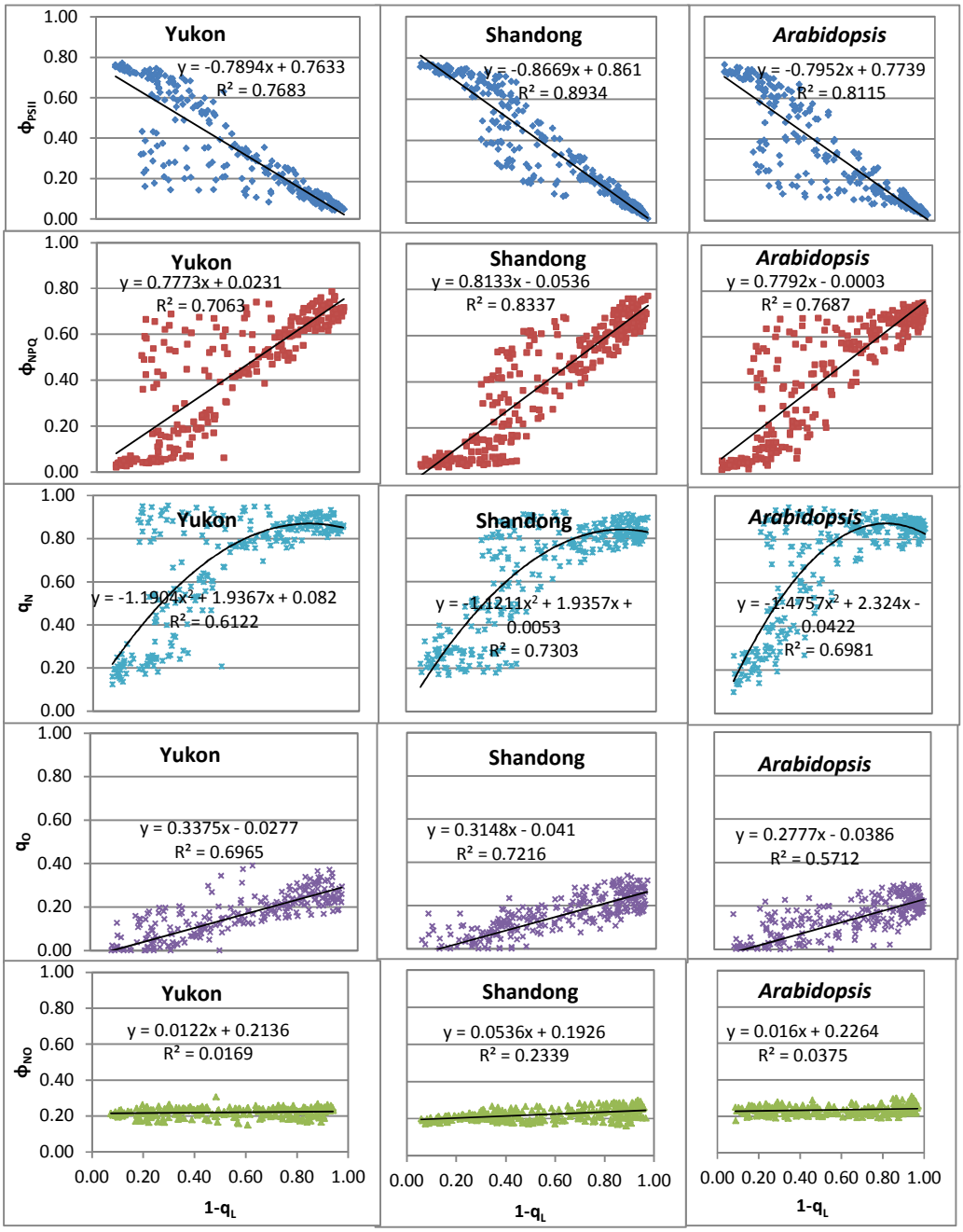
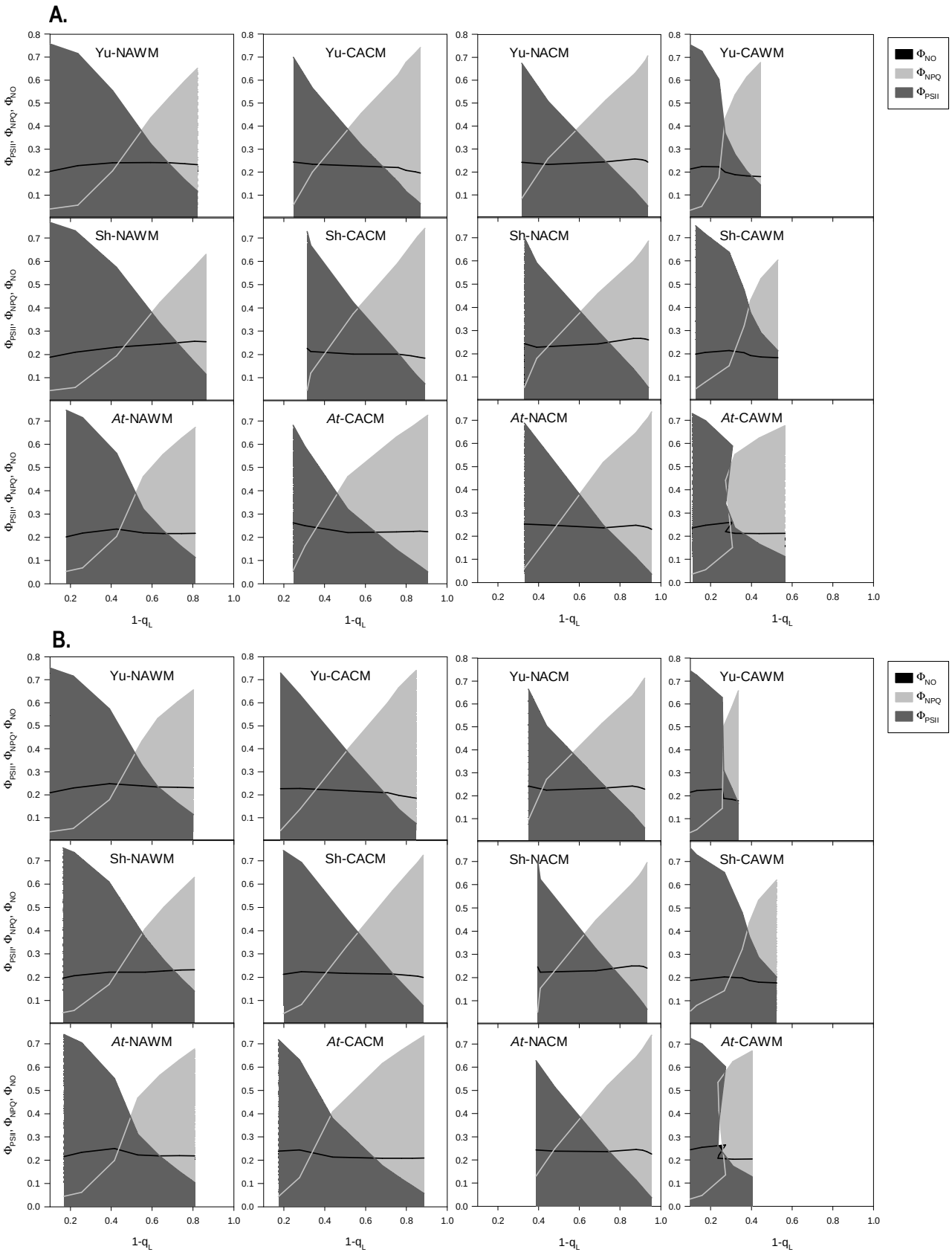


Figure 5.7. Relationship between excitation pressure ($1-q_L$) and other correlates of PSII performance. The results of Yukon, Shandong and *Arabidopsis* are pooled from various experimental conditions including different growth regimes, acclimation status, measurement temperatures and irradiances. Φ_{PSII}, PSII operating efficiency; Φ_{NPQ}, efficiency of light dependent NPQ; q_N, non-photochemical quenching coefficient; q_O, basal fluorescence quenching coefficient; Φ_{NO}, efficiency of constitutive non-photochemical energy dissipation and fluorescence.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ΦNPQ
ΦPSII
ΦPSII, ΦNPQ, ΦNO
1-qL

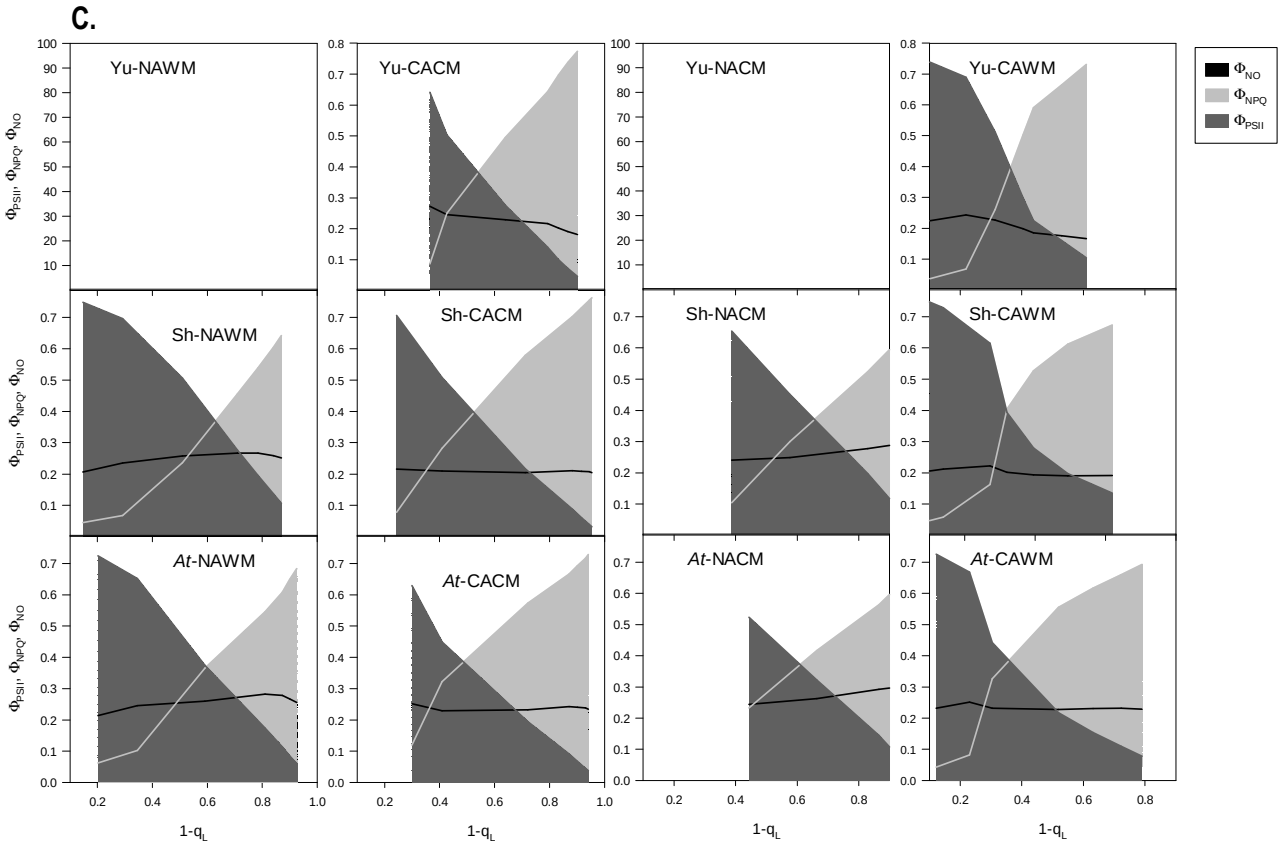

Figure 5.8. Partitioning of excitation energy as a function of excitation pressure (1-q_L) for *Thellungiella* ecotypes and *Arabidopsis*. The fraction of excitation energy flow via PSII photochemistry (Φ_{PSII}) and non-photochemical dissipation pathways (Φ_{NO} and Φ_{NPQ}) in Yukon (Yu), Shandong (Sh) and *Arabidopsis* (At) was estimated for growth under Yukon (**A.**), Shandong (**B.**) and *Arabidopsis* (**C.**) growth regimes. Plants were measured at their respective growth temperatures as well as reciprocal temperature measurements as indicated. The blank graphs of Yukon ecotype in the *Arabidopsis* growth regime denotes no results obtained due to poor growth of the ecotype in that condition. CACM, cold acclimated cold measured; CAWM, cold acclimated warm-measured; NACM, non-acclimated cold-measured; NAWM, non-acclimated warm-measured.

5.3.2 Photoinhibition and Recovery of PSII

Exposure of *Arabidopsis* and Shandong plants grown under three non-acclimating and cold-acclimating conditions to a high irradiance of 1750 µmol photons m^{-2} s^{-1} coupled with low temperature (7°C) for 4 hours resulted in differential levels of photoinhibition between the species across the growth conditions. Combined analysis of the photoinhibited values of F_v/F_m showed significant differences between the taxa *(P* < 0.001), growth regimes *(P* < 0.001) and acclimation status *(P* < 0.001). The taxa also had significant interactions with growth regimes and acclimation status (Figure 5.9).

Cold acclimation significantly enhanced the tolerance to photoinhibition. Conversely, the reduction in F_v/F_m was significantly higher (32 to 54%) in non-acclimated plants than that of cold acclimated plants (12 to 31%; Figure 5.9). It is evident that modulation of F_v/F_m is a prominent strategy of cold acclimation in both *Arabidopsis* and *Thellungiella*.

Growth conditions, especially growth irradiance seemed to be an important determinant of the tolerance of plants to photoinhibition. In general, plants grown with higher irradiance (250 µmol photons m^{-2} s^{-1} in the Yukon and Shandong growth regimes) experienced less photoinhibition than those grown under lower irradiance (100 µmol photons m^{-2} s^{-1} in the *Arabidopsis* regime). Under non-acclimating conditions, plants grown with an irradiance of 250 µmol photons m^{-2} s^{-1} exhibited a reduction of F_v/F_m in the range of 32 to 36%, while those values for plants grown with an irradiance of 100 µmol photons m^{-2} s^{-1} were 35 to 54%. Cold acclimated plants also exhibited a similar effect of growth irradiance on the extent of photoinhibition. The cold acclimated plants from a growth irradiance of 250 µmol photons m^{-2} s^{-1} had a lower extent of photoinhibition (12 to 25%) than those from the lower growth irradiance (17 to 31%; Figure 5.9).

The interactions of experimental taxa with growth regimes and acclimation status resulted in the variation in the extent of photoinhibition between the taxa. Yukon and Shandong displayed relatively more stable values of F_v/F_m with a lower extent photoinhibition than *Arabidopsis* (Figure 5.9). Under higher growth irradiance (250 µmol photons m^{-2} s^{-1}), photoinhibitory responses of *Arabidopsis* were at par with *Thellungiella*. However, when grown under lower irradiances (100 µmol photons m^{-2} s^{-1}), *Arabidopsis* showed greater susceptibility to photoinhibition than the *Thellungiella* ecotypes. For instance, with a growth irradiance of 250 µmol photons m^{-2} s^{-1}, the non-acclimated plants of all three experimental taxa underwent photoinhibition by 32 to 36% and the cold acclimated plants by 12 to 24%. Under the lower growth irradiance (100 µmol photons m^{-2} s^{-1} at *Arabidopsis* growth regime) on the other hand, *Arabidopsis* underwent photoinhibition by 54% and 31% in non-acclimated and cold-acclimated plants respectively, while the corresponding values for *Thellungiella* were 35 to 38% in non-acclimated plants and 17 to 22% in

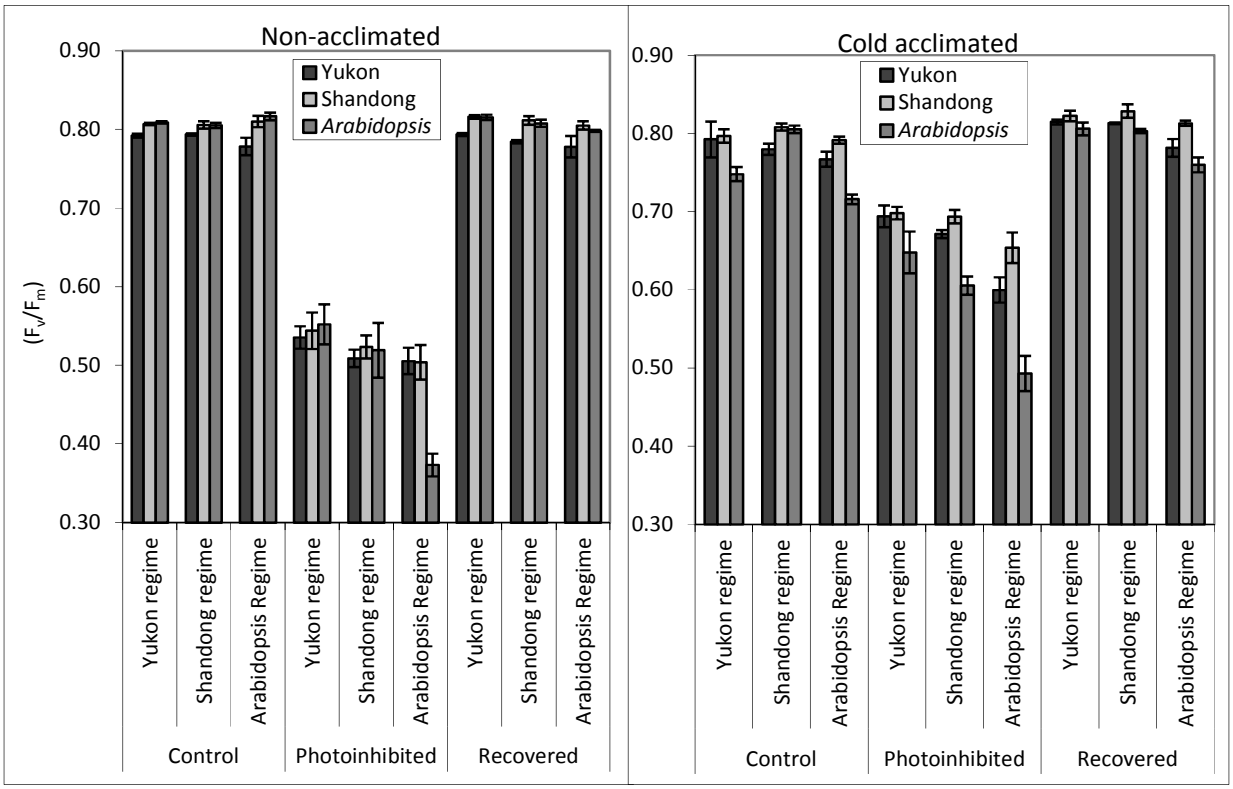

Figure 5.9. Photoinhibition and recovery monitored as changes in maximum quantum efficiency (F_v/F_m) of non-acclimated and cold acclimated *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Yukon ecotype (■); Shandong ecotype (■); *Arabidopsis* (■). Photoinhibition occurred for 4 h with a PPFD of 1700 µmol photons m^{-2} s^{-1} at 7°C. Recovery occurred at room temperature under dim light for 24 h. Values represent means ± SE (*n* = 3 to 6).

cold acclimated plants. These results suggest that *Thellungiella* ecotypes possessed greater constitutive tolerance to photoinhibition and attained better photostasis through cold acclimation than *Arabidopsis*.

After the release of photoinhibitory treatments, plants were kept at room temperature (22 to 24°C) with a low irradiance of about 30 µmol photons m^{-2} s^{-1}. After 24 hours of releasing the photoinhibitory stress, all plants fully recovered their F_v/F_m to the equivalent level of the control plants. This recovery suggests that the photoinhibition in all taxa was reversible, suggesting the effectiveness of repair of the photosynthetic apparatus.

5.3.3 Redox State of PSI and the Intersystem Electron Pool

The exposure of the leaves to far-red (FR) light resulted in an absorbance change at 820 nm ($\Delta A_{820}/A_{820}$), an indicator of the oxidation of P_{700}. The P_{700}^+ was transiently reduced with the application of saturating single-turnover (ST) flash or multiple turnover (MT) flash in the presence of background FR light. The ratio of the extent of reduction of P_{700} triggered by the MT flash to the ST flash is an indicator of the number of electrons stored in the intersystem electron transport chain (e^-/P_{700}) or intersystem electron pool size (see also Section 2.3.4.6).

Yukon, Shandong and *Arabidopsis* differed significantly ($P < 0.000$) for P_{700} oxidation within and across the growth regimes, acclimation status and measurement temperature. The taxa also displayed significant interactions ($P < 0.000$) with growth regime and cold acclimation for the extent of P_{700} oxidation (Figure 5.10). In all experimental taxa, the effect of measurement temperature on P_{700} oxidation was consistently similar. The generalized effect was that all three experimental taxa showed a consistently higher extent of P_{700} oxidation at low measurement (4.5°C) than at warm measurement (20°C) temperature (Figure 5.10). The differences between the corresponding values were significant, though the magnitudes of differences were not very high (2.9 to 13.78% on average).

Unlike the low temperature measurement, the effect of cold acclimation on P_{700} oxidation was variable between the experimental taxa (Figure 5.10). Cold acclimation enhanced the capacity of P_{700} oxidation in Yukon (3 to 68% increase) and *Arabidopsis* (22 to 98% increase) grown across all growth regimes. Shandong plants grown at a low irradiance of 100 µmol photons m^{-2} s^{-1} (*Arabidopsis* growth regime) also displayed a significant increase (61 to 77%) in P_{700} oxidation due to cold acclimation. However, when Shandong was grown under a growth irradiance of 250 µmol photons m^{-2} s^{-1} (Shandong and Yukon growth regimes) the modulating effect of cold acclimation on P_{700} oxidation disappeared (-9.5 to 0.8% change).

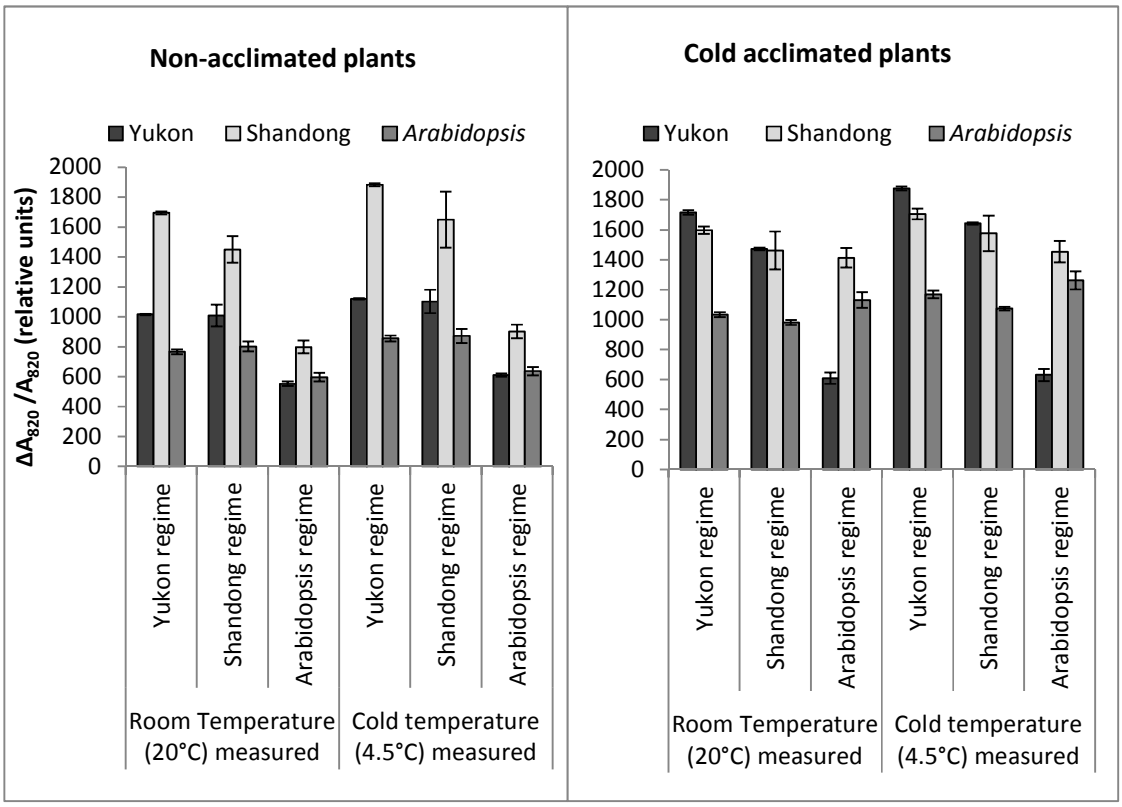

Figure 5.10. P_{700} oxidation measured as $\Delta A_{820}/A_{820}$ for non-acclimated and cold acclimated *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Yukon ecotype (■); Shandong ecotype (■); *Arabidopsis* (■). Plants were measured at warm temperature (20ºC) and low temperature (4.5ºC). Values represent means ± SE (n = 4 to 9).

Conversely, it seemed that moderate irradiance and cold acclimation independently triggered a similar effect on P_{700} redox properties in Shandong.

Compared to *Arabidopsis* growth regime, the major commonality between the Yukon and Shandong growth regimes was the level of growth irradiance (100 µmol photons m^{-2} s^{-1} versus 250 µmol photons m^{-2} s^{-1}). In all experimental taxa, an increase in growth irradiance from 100 µmol photons m^{-2} s^{-1} (*Arabidopsis* regime) to 250 µmol photons m^{-2} s^{-1} (Yukon and Shandong regime) had a significant positive effect on the extent of P_{700} oxidation under non-acclimated conditions (Figure 5.10). However, Shandong and Yukon had better response to growth irradiance in relation to P_{700} oxidation with the greater magnitude of increase (82 to 112% increase) than that of *Arabidopsis* (28 to 37% increase) under non-acclimated conditions. Similarly, cold acclimated Yukon distinguished itself with the highest response to growth irradiance with a 142 to 197% increase in P_{700} oxidation due to the change in growth irradiance from 100 µmol photons m^{-2} s^{-1} to 250 µmol m^{-2} s^{-1}. Shandong followed the trend with a moderate response (4 to 17% increase) in the amount of oxidized P_{700} with the increase in irradiance. However, cold acclimated *Arabidopsis* showed a contrasting response in that the P_{700} oxidation was 7 to 15% lower in plants grown under higher growth irradiances.

Stagnant growth of Yukon at a low irradiance of 100 µmol photons m^{-2} s^{-1} and its greater response to increased irradiance in growth and photosynthetic parameters suggested that the *Arabidopsis* growth regime was barely meeting the light compensation point for this ecotype. The growth stagnation seemed to be associated with a lower capacity for P_{700} oxidation, due presumably to the low photosynthesis. In an earlier study, the extent of P_{700} oxidation was found to be positively linked with CO_2 assimilation (Herbinson and Hedley 1993). Therefore, the P_{700} oxidation capacity in turn can have a bearing on plant growth. Except for the *Arabidopsis* growth regime, both ecotypes of *Thellungiella* outperformed *Arabidopsis* for P_{700} oxidation in all growth and measurement conditions. Non-acclimated Shandong consistently displayed higher amounts of oxidized P_{700} than both Yukon and *Arabidopsis*. Cold acclimated values of P_{700} oxidation of Yukon and Shandong were quite similar (Figure 5.10).

The oxidized P_{700} is reduced by the electrons originated mainly from the photo-oxidation of water and are conveyed through linear electron transport via PSII, the PQ pool and plastocyanin. The intersystem electron pool is also fed by other pathways such as stromal and cyclic electron transport around PSI. These properties also constitute the components of photosynthetic adjustment strategies of different taxa in response to growth regimes.

The e^-/P_{700} was found to be the product of complex interactions between the experimental taxa, growth regimes or measurement conditions. There were no consistent responses of the experimental taxa to measurement temperature, cold acclimation or growth regime for this parameter (Figure 5.11). Exceptionally higher values of e^-/P_{700} were detected in non-acclimated, warm measured Yukon in the *Arabidopsis* regime and cold acclimated, low temperature measured values of *Arabidopsis* in the Yukon regime. These observations were associated with apparent growth abnormalities. In the former case, Yukon growth was arrested due to a limitation of growth irradiance, while in the latter case, *Arabidopsis* displayed pale and stunted foliage as a combined effect of cold, longer photoperiod and high irradiance. A generalized scenario of combined analysis displayed significant effects of cold acclimation *(P* < 0.001) and measurement temperature *(P* = 0.041). However, these trends were complicated by the significant interaction of taxa with growth regime and measurement temperature. In general, cold acclimation resulted in an increase of the intersystem electron pool in Shandong (7 to 86% increase) and *Arabidopsis* (31 to 126% increase), while for Yukon this trend was not amenable for generalization due to interacting effect of growth regime and measurement temperature. Barring few exceptional observations, measurement at low temperature caused the lowering of e^-/P_{700} in all three taxa across all growth regimes. The differences in the low temperature-measured values of Yukon between the non-acclimated and cold acclimated plants were relatively smaller.

The trend of the effect of growth irradiance on e^-/P_{700} is variable between the experimental taxa. Yukon and *Arabidopsis* displayed interactions between growth regime, acclimation and measurement temperature, making it difficult to discern the effect of growth irradiance. However, for Shandong, e^-/P_{700} values showed an increasing trend (10 to 90% increase) as the result of increase in growth irradiance from 100 µmol photons m^{-2} s^{-1} to 250 µmol photons m^{-2} s^{-1} (Figure 5.11).

5.3.4 Photosynthetic Pigmentation

Growth conditions affect variables such as leaf area and thickness that in turn has an effect on the leaf pigmentation. Moreover, leaf pigment composition also reflects the acclimation and adaptation strategies of plants in response to various environmental factors that affect photosynthesis. A combined analysis of various photosynthetic pigmentation parameters showed significant difference between the taxa under study across growth conditions shaped by individual environmental factors and their interplay.

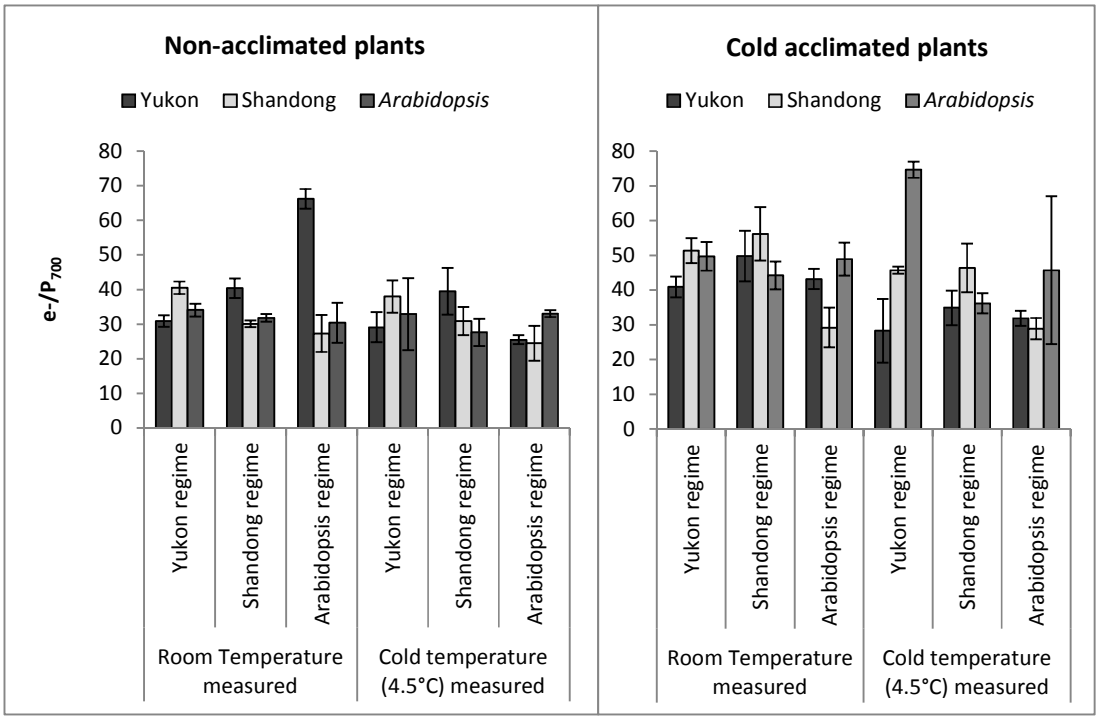

Figure 5.11. Pool size of electrons in the intersystem chain (e^-/P_{700}) for non-acclimated and cold acclimated *Thellungiella* ecotypes and *Arabidopsis* developed under the growth regimes indicated. Yukon ecotype (■); Shandong ecotype (■); *Arabidopsis* (■). Plants were measured at warm temperature (20ºC) and low temperature (4.5ºC). Values represent means ± SE (*n* = 4 to 9).

Combined analysis of photosynthetic pigmentation parameters showed a significant difference in chlorophyll content per unit leaf fresh weight (ChlW) between the taxa *(P* < 0.001), between the growth regimes *(P* = 0.037) and between the cold acclimation status *(P* < 0.001) of the plants. For this parameter, significant two or three-way interactions were found between the genotypic and environmental factors: taxa by growth regime *(P* = 0.014), taxa by acclimation *(P* < 0.001), growth regime by cold acclimation *(P* = 0.04) and ecotype by growth regime by cold acclimation *(P* = 0.005). Chlorophyll content per unit leaf area (ChlLA) also varied significantly between the taxa *(P* < 0.001), across the growth regimes *(P* < 0.001) and cold acclimation status *(P* = 0.036) along with a significant two and three way interactions between taxa, growth regime and acclimation status *(P* < 0.001 to 0.05; Table 5.4). This suggests that Chl content is a function of individual taxa that respond differentially across the growth regimes shaped by growth temperature, irradiance and photoperiod.

Similarly, carotenoid content per unit leaf weight (CarW) differed significantly between the taxa *(P* < 0.001) and between the acclimation status *(P* < 0.001). However, no significant difference was observed for this parameter across the growth regimes *(P* = 0.188). CarW also appeared to be affected significantly by the interactions between the taxa and growth regime *(P* = 0.004), between taxa and acclimation status *(P* < 0.001), between growth regime and cold acclimation *(P* = 0.008) and between ecotype, growth regime and acclimation status *(P* = 0.039). Carotenoid content per unit leaf area (CarLA) also displayed highly significant difference between the taxa, growth regimes and acclimation status *(P* < 0.001 for all factors) with significant two and three way interactions between the genotypic and environmental factors *(P* < 0.001 to 0.015) (Table 5.4).

In the overall analysis, the taxa did not vary significantly in the chlorophyll *a:b* ratio (Chl *a:b*; P = 0.91), but significant alterations in the values were observes across the growth regimes *(P* < 0.001) and acclimation status *(P* = 0.004). For this parameter, significant interactions were found between taxa and growth regime *(P* < 0.001), between ecotype and acclimation status *(P* < 0.001) and between ecotype, growth regime and acclimation status *(P* = 0.002). However, no significant interaction was observed between growth regime and acclimation status *(P* = 0.099) in Chl *a:b* (Table 5.4).

Unlike other pigmentation parameters, the chlorophyll:carotenoid ratio (Chl:Car) did not differ significantly between the taxa *(P* = 0.389) and across growth regimes *(P* = 0.06). However, acclimation status altered the ratio significantly *(P* < 0.001), with significant interactions between the ecotype and acclimation (P=0.018), between taxa and growth regimes *(P* = 0.019), and between taxa, growth regimes and acclimation status *(P* = 0.001).

Table 5.3. Comparison of photosynthetic leaf pigmentation in Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown at three different regimes

Parameter	Taxa	Growth regime					
		Yukon		Shandong		*Arabidopsis*	
		Non-acclimated	Cold acclimated	Non-acclimated	Cold acclimated	Non-acclimated	Cold acclimated
ChlW (µg mg^{-1})	Yukon	2.4 ± 0.07$^{a\,A}$	1.9 ± 0.08$^{a\,B}$	2.4 ± 0.10$^{a\,A}$	1.9 ± 0.05$^{a\,B}$	2.4±0.29$^{a\,A}$	2.2 ± 0.04$^{a\,A}$
	Shandong	2.2 ± 0.02$^{a\,A}$	1.6 ± 0.03$^{b\,B}$	2.2 ± 0.11$^{a\,A}$	1.6 ± 0.02$^{b\,B}$	2.5±0.03$^{a\,A}$	1.1 ± 0.21$^{c\,B}$
	Arabidopsis	1.3 ± 0.05$^{b\,A}$	1.3 ± 0.06$^{c\,A}$	1.2 ± 0.07$^{b\,A}$	1.5 ± 0.03$^{c\,A}$	1.7±0.05$^{b\,A}$	1.6 ± 0.07$^{b\,A}$
CarW (µg mg^{-1})	Yukon	0.36 ± 0.009$^{a\,B}$	0.46 ± 0.010$^{a\,A}$	0.40 ± 0.005$^{a\,B}$	0.49 ± 0.012$^{a\,A}$	0.41 ± 0.047$^{a\,A}$	0.48 ± 0.022$^{a\,A}$
	Shandong	0.43 ± 0.003$^{a\,A}$	0.35 ± 0.010$^{b\,B}$	0.34 ± 0.016$^{b\,A}$	0.37 ± 0.008$^{b\,A}$	0.38 ± 0.008$^{a\,A}$	0.30 ± 0.053$^{b\,A}$
	Arabidopsis	0.23 ± 0.010$^{b\,B}$	0.33 ± 0.010$^{b\,A}$	0.20 ± 0.013$^{c\,B}$	0.29 ± 0.012$^{c\,A}$	0.29 ± 0.021$^{b\,A}$	0.33 ± 0.013$^{b\,A}$
ChlLA (µg cm^{-2})	Yukon	64.5 ± 5.22$^{a\,A}$	63.3 ± 1.35$^{b\,A}$	64.1 ± 2.45$^{a\,A}$	65.7 ± 4.08$^{a\,A}$	39.0 ± 2.2$^{b\,B}$	62.0 ± 2.48$^{a\,A}$
	Shandong	72.2 ± 2.69$^{a\,A}$	71.3 ± 2.67$^{a\,A}$	71.3 ± 4.05$^{a\,A}$	78.1 ± 4.82$^{a\,A}$	61.7 ± 1.84$^{a\,A}$	32.8 ± 5.80$^{b\,B}$
	Arabidopsis	36.1 ± 2.38$^{b\,A}$	38.3 ± 2.35$^{c\,A}$	26.8 ± 1.26$^{b\,B}$	46.8 ± 1.46$^{b\,A}$	32.9 ± 1.26$^{c\,B}$	42.6 ± 1.30$^{b\,A}$
CarLA (µg cm^{-2})	Yukon	9.9 ± 0.64$^{b\,B}$	15.4 ± 0.58$^{a\,A}$	10.8 ± 0.64$^{a\,B}$	17.3 ± 0.57$^{a\,A}$	6.7 ± 0.32$^{b\,B}$	13.5 ± 0.36$^{a\,A}$
	Shandong	14.1 ± 0.44$^{a\,A}$	15.4 ± 0.43$^{a\,A}$	11.2 ± 0.73$^{a\,B}$	18.1 ± 1.16$^{a\,A}$	9.4 ± 0.14$^{a\,A}$	8.9 ± 1.47$^{b\,A}$
	Arabidopsis	6.5 ± 0.34$^{c\,B}$	9.5 ± 0.43$^{b\,A}$	4.4 ± 0.24$^{b\,B}$	9.0 ± 0.27$^{b\,A}$	5.5 ± 0.19$^{c\,B}$	8.7 ± 0.23$^{b\,A}$
Chl *a*:*b*	Yukon	3.1 ± 0.13$^{c\,B}$	4.9 ± 0.50$^{a\,A}$	3.3 ± 0.29$^{a\,B}$	4.8 ± 0.06$^{a\,A}$	2.8 ± 0.15$^{b\,A}$	3.8 ± 0.39$^{a\,A}$
	Shandong	4.7 ± 0.12$^{a\,A}$	4.2 ± 0.10a$^{b\,A}$	3.6 ± 0.20$^{a\,B}$	4.8 ± 0.22$^{a\,A}$	3.2 ± 0.14$^{b\,A}$	2.2 ± 0.30$^{b\,B}$
	Arabidopsis	3.9 ± 0.05$^{b\,A}$	3.7 ± 0.37$^{b\,A}$	4.0 ± 0.08$^{a\,A}$	3.3 ± 0.24$^{b\,B}$	3.9 ± 0.22$^{a\,A}$	4.1 ± 0.11$^{a\,A}$
Chl:Car	Yukon	6.5 ± 0.21$^{a\,A}$	4.1 ± 0.16$^{a\,B}$	6.0 ± 0.28$^{a\,A}$	3.8 ± 0.14$^{b\,B}$	5.9 ± 0.41$^{a\,A}$	4.6 ± 0.29$^{a\,A}$
	Shandong	5.1 ± 0.04$^{b\,A}$	4.6 ± 0.12$^{a\,A}$	6.4 ± 0.27$^{a\,A}$	4.3 ± 0.10$^{b\,B}$	6.6 ± 0.16$^{a\,A}$	3.7 ± 0.20$^{b\,B}$
	Arabidopsis	5.5 ± 0.23$^{b\,A}$	4.0 ± 0.24$^{a\,B}$	6.1 ± 0.05$^{a\,A}$	5.2 ± 0.28$^{a\,A}$	6.0 ± 0.28$^{a\,A}$	4.9 ± 0.08$^{a\,B}$

Data are expressed on a leaf FW basis (W) or a leaf area basis (LA)
The data were analyzed using ANOVA and the means were separated using Fisher's individual error rate
Values followed by small case letters along columns are significant within growth regime and the capital letters along the rows denote the significant difference of a taxa across the acclimation state
Values represent means ± SE (n = 3 to 5)
ANOVA, anaysis of variance; Chl, chlorophyll; Car, carotenoid; SE, standard error

The significant interactions between the taxa with growth regimes and measurement temperature imply that the taxa adopt differential photosynthetic adjustment strategies in response to changes in growth regimes and measurement conditions. The interactions between the environmental variables show that the combined effect of individual environmental components is different from that of additive effects of individual factors in photosynthetic pigmentation.

5.3.4.1 Effect of Cold Acclimation on Pigmentation Across Growth Regimes

Cold acclimation had differential effects on photosynthetic pigmentation between the taxa. Upon cold acclimation for 3 weeks, Yukon showed a significant decrease in ChlW under the Yukon and Shandong regimes, but there was no significant change in this parameter in the *Arabidopsis* regime. Shandong consistently displayed significant decrease in ChlW upon cold acclimation across all three growth regimes. On the other hand, *Arabidopsis* showed no significant change in ChlW upon cold acclimation under all three growth regimes. Chlorophyll content per unit leaf area (ChlLA) remained more or less stable without any significant change due to cold acclimation in all three taxa across growth regimes (Table 5.4).

Yukon and *Arabidopsis* showed similar trends in CarW due to cold acclimation, with significant increases in the Yukon and Shandong regimes, while having no significant change in *Arabidopsis* regime. Contrarily, Shandong did not show significant changes in CarW upon cold acclimation across all regimes. On a leaf area basis, CarLA increased significantly upon cold acclimation across all three growth regimes in Yukon and *Arabidopsis*. Again, Shandong set itself apart from other counterparts with no significant change in CarLA upon cold acclimation under the Yukon and *Arabidopsis* regimes, though there was a significant increase in CarLA of this ecotype upon cold acclimation under the Shandong regime (Table 5.4).

The taxa did not show any definite trends in Chl *a:b* upon cold acclimation. Yukon showed a significant increase in Chl *a:b* in the Yukon and Shandong growth regimes while having no significant change in the *Arabidopsis* regime. Contrarily, upon cold acclimation, Shandong underwent a significant increase in Chl *a:b* in the Shandong regime, while displaying no significant change in this parameter in Yukon regime and a significant decrease in the *Arabidopsis* regime. On the other hand, cold acclimation brought about no significant change in Chl *a:b* in *Arabidopsis* in the Yukon and *Arabidopsis* regimes and surprisingly decreased the value of this parameter in the Shandong regime. A generalized observation of cold acclimation was a decrease in the Chl:Car ratio in all taxa across all three growth regimes. However, in some of the cases, the change in the ratio was not statically significant (Table 5.4).

With only a few exceptions, the *Thellungiella* ecotypes showed a significantly higher content of both chlorophyll and carotenoid pigments both on a per unit weight and per unit leaf area basis across all growth regimes under both acclimated and non-acclimated conditions. Again with few exceptions, Yukon seemed to be significantly higher than or at par with Shandong for the pigment content considered. Except for the *Arabidopsis* regime, Shandong had either a significantly higher or similar Chl *a:b* ratio across all regimes, while the relationship was opposite in the *Arabidopsis* regime. Contrarily, for the Chl:Car ratio, Shandong appeared to be significantly lower than or equivalent to Yukon and *Arabidopsis* across all growth regimes. In most cases, *Arabidopsis* and Yukon displayed similar level of the Chl:Car ratio across all growth regimes (Table 5.4). Some exceptions in the relative content of the photosynthetic pigment parameters suggest that there was interaction between the taxa and the growth regimes.

The above results show that photosynthetic pigmentation parameters differed across growth regimes with significant interactions between the taxa and the growth regimes. However, due to the multi-factor variation in the growth regimes including the temperature, irradiance and photoperiod, the results did not discern the effects of individual factors on the pigmentation parameters.

5.4 Discussion

Photosynthesis is highly sensitive to environmental variability and adaptation to any ecological niche requires dynamic functional flexibilities and modulation of the photosynthetic machinery in response to environmental cues (Eberhard et al. 2008). This demands a tightly regulated energy balancing mechanism of photosynthetic, and perhaps even respiratory metabolism (Raghavendra and Padmasiri 2003). Because of their extremophillic ecological background and proven tolerance to more severe stress conditions than *Arabidopsis*, it was expected that *Thellungiella* would differ from *Arabidopsis* in photosynthetic properties, which are highly sensitive to environmental conditions and prime target of stress.

The aspects considered in this study included correlates of PSI and PSII performance and photosynthetic pigmentation. The experimental variables included six growth regimes differing in irradiance, temperature and photoperiod and measurement conditions that differed in irradiance and temperature. This experimental design provided multiple scenarios for comparison of photosynthetic modulation in the experimental taxa, including the partitioning of excitation energy into photochemical and non-photochemical components. Firstly, the results from the non-acclimated warm-measured (NAWM) treatment revealed the photosynthetic responses of plants under reference control conditions. Secondly, the results from cold acclimated low temperature-measured (CACM) plants represented photosynthetic modulation under a cold

acclimating environment. Thirdly, the results from non-acclimated low temperature-measured (NACM) plants characterized the photosynthetic responses under a cold-shock. Presumably, the NACM treatment with increasing irradiance represents a condition of intensifying excitation pressure with a growing disparity between the harvesting and utilization of light energy by the photosynthetic apparatus (Gray et al. 1996; Ensminger et al. 2006). In corollary, the results from cold acclimated warm-measured (CAWM) plants typify the photosynthetic responses upon the instantaneous relief from low temperature (or the relaxation of excitation pressure).

Our results show that there are not only inter-generic differences between *Arabidopsis* and *Thellungiella*, but also inter-ecotypic differences between Yukon and Shandong in photosynthetic parameters.

5.4.1 Similar Trends but Quantitative Differences in PSII Performance Indicators of *Thellungiella* and *Arabidopsis*

Thellungiella and *Arabidopsis* plants grown under various conditions showed comparable trends in PSII performance indicators. As was anticipated, a generalized pattern of each ecotype across all growth conditions was that measurements at low temperature caused significant increase in excitation pressure with a concomitant down-regulation of Φ_{PSII} and ETR_{PSII}, and consequently saturation of photosynthesis at lower irradiance levels. At the same time, the photosynthetic down-regulation was also associated with up-regulation of non-photochemical quenching parameters. It is already established that low temperature causes membrane rigidification and decrease in enzyme kinetics leading to the down-regulation of electron transfer and carbon assimilation reactions (Ruelland et al. 2009). An examination of electron transport rate and pattern of energy partitioning revealed that photochemical down-regulation with concomitant up-regulation of NPQ was a common manifestation of cold acclimation in both *Thellungiella* ecotypes and *Arabidopsis*. At respective growth temperatures, the light response curves of cold acclimated plants positioned invariably at lower levels and light saturation occurred at lower PPFDs than that of non-acclimated control plants. On the other hand, when plants were instantaneously exposed to temperatures that contrasted with their respective growth temperatures, cold acclimated plants out-performed the non-acclimated counterparts with regard to PSII photochemistry. Instantaneous exposure of cold acclimated plants to warm temperature resulted in the thermal augmentation of PSII performance characterized by te relaxation of excitation pressure, a greater fraction of excitation energy partitioned to photochemistry and a concomitant reduction of NPQ over a wide range of PPFDs. On the other hand, non-acclimated plants upon

instantaneous exposure to low measuring temperature displayed a significant depression in the light response curves of photochemical indicators, suggesting the inhibition of photosynthesis. Growth regime also triggered conspicuous effects on photosynthetic light response curves. Compared to the plants grown at low irradiance (*Arabidopsis* growth regime), the plants grown under higher irradiances (Yukon and Shandong growth regimes) underwent a down-shift in the trend of excitation pressure coupled with the upward shifts in light response curves for PSII operating efficiency and electron transport.

Amidst the common general trends of taxonomic responses to environmental variables, *Thellungiella* and *Arabidopsis* differed from each other in the relative magnitude of PSII performance parameters. In most instances of the experimental settings, Shandong stood superior to Yukon and *Arabidopsis* in PSII performance indicators, while Yukon held an intermediate position followed closely by *Arabidopsis* at the lower profile. This generalization was more applicable after the exclusion of results from the *Arabidopsis* growth regime that was proven to be a sub-normal growth condition for the Yukon ecotype. Both the Yukon and Shandong ecotypes of *Thellungiella* were able to cold acclimate without affecting their potential quantum efficiencies, while *Arabidopsis* acclimated to low growth temperature by lowering the maximum quantum efficiency of PSII photochemistry by about 5 to 10%. These observations corroborate with an earlier finding by Savitch et al. (2001) that showed incomplete recovery of photosynthetic capacity of *Arabidopsis* upon cold acclimation. In the range of experimental PPFDs, Shandong underwent a relatively larger fraction of excitation energy partitioning towards PSII photochemistry compared to that of Yukon and *Arabidopsis*, while the later displayed more or less similar responses. Compared to Yukon and *Arabidopsis*, the Shandong ecotype showed higher rates of ETR_{PSII} on the light response curve under all experimental conditions, with less intensity of ETR_{PSII} depression of non-acclimated plants due to cold-shock and a greater magnitude of thermal augmentation of ETR_{PSII} for cold acclimated plants due to exposure to warm measuring temperatures (Table 5.2). A recent comparative study by Stepien and Johnson (2009) also showed that Shandong had higher ETR_{PSII} than *Arabidopsis* under both normal and salt-stressed conditions. Shandong also displayed less intensity of the effects of cold-shock on excitation pressure and non-photochemical quenching parameters. On the other hand, the acclimative gain from the cold-shock level and thermal relaxation due to the warming effect were relatively lower in Shandong than that of *Arabidopsis*. These are the indication that Shandong has better photosynthetic stability. The Yukon and *Arabidopsis* exhibited contrasting effects of cold-shock on non-photochemical quenching parameters. The Yukon ecotype displayed highest sensitivity of overall non-photochemical quenching (q_N) while *Arabidopsis* showed highest sensitivity of antenna quenching (q_O) to the cold-shock (Table 5.2).

Thellungiella and *Arabidopsis* also differed in photoinhibition of PSII. Yukon and Shandong displayed relatively more stable values of F_v/F_m in response to photoinhibitory treatments than *Arabidopsis*. When grown under higher irradiance (250 µmol photons m^{-2} s^{-1}), photoinhibitory responses of *Arabidopsis* were at par with *Thellungiella*. However, when grown under lower irradiance (100 µmol photons m^{-2} s^{-1}), *Arabidopsis* showed greater susceptibility to photoinhibition than the *Thellungiella* ecotypes. The relative resistance to photoinhibition was found to be associated with carotenoid content in the leaves since these parameters were negatively correlated. The photoprotective role of carotenoids is well established (Bartley and Scolnik 1995).

5.4.2 Differential Responses of *Thellungiella* and *Arabidopsis* to Excitation Pressure

Photochemistry and the non-photochemical dissipation of energy are competing processes. A high degree of determination of regression of the photochemical and non-photochemical quenching parameters on $1\text{-}q_L$ (Figure 5.7) suggested the later to be an important link in photosynthetic processes. The scatter plots and regression parameters of Shandong, Yukon and *Arabidopsis* showed that Shandong is quantitatively more responsive to excitation pressure for Φ_{PSII} and Φ_{NPQ} , Yukon for q_O and *Arabidopsis* for q_N (Figure 5.7). It has already been postulated that the energy partitioning through these competing pathways is regulated by the environmental signals perceived by photosynthesis itself through the redox state of photosynthetic electron transport components (Ensminger et al. 2006; Bräutigam et al. 2009).

The above observations led to the consideration that excitation pressure may serve as the unifying determinant of the energy partitioning. To examine the pattern of energy partitioning in response to excitation pressure, the results of energy fractionation from different experimental conditions were plotted against the excitation pressure (Figure 5.8). The portrait of energy partitioning against excitation pressure revealed a complex nature of energy fractionation that cannot be ascribed to short-term changes in excitation pressure. However, the results suggested that an excitation pressure of around 0.5 is critical for relative predominance of Φ_{PSII} or Φ_{NPQ}. When measured at growth irradiance, the intersection of Φ_{PSII} or Φ_{NPQ} curves occurred between excitation pressures of 0.49 and 0.62 in non-acclimated plants and between 0.42 to 0.56 in cold acclimated plants. *Arabidopsis* displayed relatively lower Φ_{PSII} or higher Φ_{NPQ} at a given excitation pressure than *Thellungiella* ecotypes. At reciprocal measurement temperatures, cold acclimated plants displayed a higher sensitivity of energy partitioning patterns to the changes in excitation pressure than the non-acclimated plants. This suggests that cold acclimation results in the development of

more responsive mechanisms to detect changes in environmental conditions, thereby enabling them to adjust the pattern of excitation energy partitioning.

Considering the extremophillic adaptation of *Thellungiella* in contrast with the glycophytic adaptation of *Arabidopsis*, it was anticipated that *Thellungiella* would possess better resiliency of PSII performance especially under low temperature conditions. However, Shandong only displayed minor quantitative differences from *Arabidopsis*, while Yukon appeared fairly similar to *Arabidopsis*. It may be because of the fact that the treatments imposed in the experiments were within the adaptive range of *Arabidopsis* (see Section 2.1.2). Therefore, further comparative studies between *Thellungiella* and *Arabidopsis* with more extreme environmental conditions would be tempting to unravel the divergence of glycophytic and extremophillic photosynthetic adaptation.

5.4.3 Yukon, Shandong and *Arabidopsis* Show Divergent Trends in PSI Performance

PSI has crucial role in balancing the phosphorylating and reducing potentials of cellular metabolism (Eberhard et al. 2008). Oxidation of P_{700}, an indicator of PSI activity was measured as the far-red light induced absorbance change at 820 nm ($\Delta A_{820}/A_{820}$). The redox state of P_{700} reflects the metabolic condition of chloroplast including the availability of the electron acceptors such as $NADP^+$, the extent of alternative electron transfer pathways around PSI, the redox state of ferredoxin pool and electron transfer from PSII (Herbinson and Hedley 1993). These properties, in turn, are a function of genotype, environmental variables and their interactions. Oxidized P_{700} is reduced by electrons mainly originating from the photooxidation of water which are conveyed through linear electron transport via PSII, the PQ pool and plastocyanin. The intersystem electron pool is also fed by other pathways (Asada et al. 1992). These properties constitute components of photosynthetic adjustment strategies of plants in response to environmental conditions (Bukhov et al. 2001).

Yukon, Shandong and *Arabidopsis* displayed divergent patterns of P_{700} oxidation with interactions between the taxa and environmental variables. The difference in the P_{700} oxidation was accompanied with active electron transport from PSII, as evidenced by full reduction of oxidized P_{700} by multiple turn-over flashes. Except for the case of Yukon in the light-limited condition of the *Arabidopsis* growth regime, *Thellungiella* ecotypes displayed a significantly higher amount of oxidizable P_{700} than *Arabidopsis* under all conditions and acclimation status (Figure 5.10). Under non-acclimated conditions, both ecotypes of *Thellungiella* and *Arabidopsis* showed a growth irradiance-dependent response of P_{700} oxidation. However, both ecotypes of *Thellungiella* contrasted with *Arabidopsis* in having a higher magnitude of growth

irradiance response. With the increase in irradiance from 100 µmol photons m^{-2} s^{-1} (*Arabidopsis* growth regime) to 250 µmol photons m^{-2} s^{-1} (Yukon and Shandong growth regimes), the *Thellungiella* ecotypes responded with a minimum of an 82% increase in P_{700} oxidation whereas the corresponding increase in P_{700} oxidation of *Arabidopsis* was approximately 28%. Non-acclimated Shandong consistently displayed higher amounts of oxidized P_{700} than both Yukon and *Arabidopsis* (Figure 5.10). Yukon significantly outperformed *Arabidopsis* under a growth irradiance of 250 µmol photons m^{-2} s^{-1}. However, it is notable that Yukon had the lowest oxidizable P_{700} in the *Arabidopsis* growth regime (Figure 5.10) that presumably barely met light compensation point of this ecotype, resulting in stagnant growth and eventual collapse of plants after 4 weeks of germination. This shows an interesting relationship between P_{700} oxidation and plant growth. In fact, an earlier study has shown the relationship between the oxidizable P_{700} and CO_2 assimilation in plants (Harbinson and Hedley 1993).

Yukon and Shandong also contrasted each other and with *Arabidopsis* in the cold acclimated response of P_{700}. Although cold acclimated *Arabidopsis* had lower oxidizable P_{700} than *Thellungiella*, it displayed the greatest acclimatory change in this parameter due to cold acclimation. The Yukon ecotype also underwent a significant increase in P_{700} oxidation as a cold acclimatory response. However, Shandong showed a growth irradiance-dependent acclimation pattern. In this ecotype, there was marked increase in oxidizable P_{700} as a result of acclimation in the *Arabidopsis* growth regime (low irradiance), but no acclimatory response in the Yukon and Shandong growth regimes (higher irradiance) (Figure 5.10). This is presumably due to enhancement of PTOX activity of Shandong at higher irradiance, rendering acclimatory adjustment in PSI unnecessary in the given environmental conditions. This argument can be supported by the fact that salinity treatment triggered a significant up-regulation of PTOX with concomitant increase in ETR_{PSII} in Shandong, while there was no such up-regulation of PTOX and ETR_{PSII} was reduced in *Arabidopsis* (Stepien and Johnson 2009). In this study, higher irradiance could have triggered such a response in Shandong. Presumably, the irradiance was not high enough for triggering PTOX or other alternative mechanisms in Yukon, as this ecotype had more sensitive growth plasticity to irradiance suggesting a higher irradiance requirement for optimal growth than to that of Shandong and *Arabidopsis*.

For the pool size of electrons in the intersystem chain (e^-/P_{700}), there were no consistent responses of the experimental taxa to measurement temperature, cold acclimation or growth regime. In general, Shandong showed a trend of increasing e^-/P_{700} with increase in irradiance and also due to cold acclimation. In the Shandong ecotype, there was some correspondence between e^-/P_{700} and ETR_{PSII} that explains partly what results in the increased e^-/P_{700} size. However, Yukon did not display any definite trend of e^-/P_{700} in

response to environmental variables, while *Arabidopsis* responded to cold acclimation with an increase in e^-/P_{700}, without any definite trend across the growth regimes (Figure 5.11). Moreover, Yukon and *Arabidopsis* plants that had perturbed phenotypes in two contrasting growth regimes and acclimation status showed an exceptional escalation of e^-/P_{700} for contrasting measurement temperatures. In Yukon, those observations were associated with warm temperature measurements of the plants from the non-acclimated *Arabidopsis* growth regime where plant growth was severely arrested due presumably to a deficit of irradiance to meet the metabolic demand of the plants. In *Arabidopsis*, on the other hand, the exceptional results were associated with low temperature measurement of cold acclimated plants from the Yukon growth regime, where plant phenotypes were chlorotic in nature. In fact, cold acclimated *Arabidopsis* had significantly lower chlorophyll contents in the Yukon growth regime compared to other growth regimes (Table 5.4). These results showed that there was a complex interaction between the taxa and environmental variables that determine the relative predominance of electron flux in the intersystem pool of electrons. Further studies are needed to examine the predominance of electron sinks under different environmental conditions to unravel this mystery.

5.4.4 Differential Photosythetic and Photoprotective Stratagies Indicated by Photosynthetic Pigmentation

Chlorophyll and carotenoids are integral components of the photosynthetic machinery. Relative content of these pigments determines both the magnitude of energy transformation and the competence of photoprotection. Chlorophyll content is the proxy indicator of photosynthetic competence of plants, while carotenoid content reflects the photoprotective potential in plant tissues. Therefore, leaf pigment composition reflects the acclimation and adaptation strategies of plants in response to various environmental factors that affect photosynthesis (Nishio 2000).

A combined analysis of various photosynthetic pigmentation parameters showed significant differences between the taxa under study across growth conditions shaped by individual environmental factors and their interplay. Significant two- or three-way interactions between the taxa, growth regimes and acclimation status indicated that Yukon, Shandong and *Arabidopsis* differentially respond to environmental variables by adjusting their photosynthetic machinery. The interactions between the environmental variables suggested that the combined effects of individual environmental components are different from that of additive effects of individual factors in photosynthetic pigmentation.

With a few exceptions arising from the interactions between the experimental factors, *Thellungiella* ecotypes showed a significantly higher content of both chlorophyll and carotenoid pigments than *Arabidopsis* both on a per unit FW and unit LA basis across all growth regimes under both cold acclimated and non-acclimated conditions. The *Thellungiella* and *Arabidopsis* also displayed contrasting strategies of balancing photosynthetic and photoprotective pigmentation. All three taxa acclimated to low temperature by lowering the Chl:Car ratio, but *Thellungiella* ecotypes reduced the chlorophyll content, while *Arabidopsis* increased the carotenoid content to result in the equivalent cold acclimated Chl:Car ratio. A study by Stepien and Johnson (2009) showed that leaves of *Thellungiella* contained approximately a 30% higher chlorophyll content than that of *Arabidopsis* and the pigment disparity between the species increased upon salinity treatment. In an earlier study, M'rah et al. (2006) also found similar results. These results indicate that *Thellungiella* ecotypes not only maintain greater photosynthetic and photoprotective potential than *Arabidopsis*, they also modulate photosynthetic and photoprotective strategies more dynamically in response to environmental conditions. These observations correspond well with the significantly higher capacity of *Thellungiella* to oxidize P_{700} and have higher light saturation points with respect to Φ_{PSII}, ETR_{PSII}, $1\text{-}q_L$. and Φ_{NPQ}. The increase in light saturation levels of PSII performance parameters due to cold acclimation, herein termed as PSII acclimation capacity, was also higher in *Thellungiella*. These features reflect the extremophillic ecological background of *Thellungiella* that requires a more dynamic mechanism to balance photosynthetic and photoprotective potentials, in contrast to the glycophytic adaptation of *Arabidopsis*.

Yukon and Shandong also showed differential pigment modulating properties in response to environmental variables. Shandong seemed to have the strategy of more abundant light interception and energy transformation, with a higher content of both chlorophyll and carotenoids per unit LA than that of Yukon (Table 5.4). The higher pigment content of Shandong relates to the significantly higher ETR_{PSII} of this ecotype compared to that of Yukon ecotype (Figure 5.3). These ecotypes also differed in cold acclimation strategies in that Yukon had a significant increase Chl *a:b* due to cold acclimation across all growth regimes (Table 5.4), but Shandong showed variable trends with no appreciable alteration in Chl *a:b* (Table 5.4). This indicates that Yukon tends to cold acclimate by reducing photosynthetic light harvesting, while Shandong undergoes proportionate decrease in both pigments (Chl *a* + *b*) during cold acclimation. These differences in cold acclimation strategies are also reflected in the differential trends of P_{700} oxidation in Yukon and Shandong (Figure 5.10).

5.5 Conclusions

Having evolved in contrasting ecological backgrounds, *Arabidopsis*, Yukon and Shandong presumably respond differentially to environmental variability. These taxa showed differential physiological plasticity to growth temperature and irradiance. Photosynthesis is a highly sensitive metabolic processes to the environmental alterations and these processes may reflect their ecological background in stress-response strategies. This study has shown that these taxa interact differentially to instantaneous (measuring) and long-term (acclimation) changes in temperature and irradiance with their photosynthetic behavior. *Thellungiella* contained a higher amount of photosynthetic pigments, showed higher oxidation of P_{700} and possessed more resilient photoprotective mechanism than that of *Arabidopsis*. Shandong distinguished itself from its relative counterparts by having greater PSII operating efficiency and P_{700} oxidizing capacity, while Yukon set itself apart with greater growth plasticity to irradiance. Due to these unique properties, *Thellungiella* can be used as a useful complementary model plant for unraveling the mechanism of modulation of photosynthetic functional flexibilities and acclimatory adjustments that enable them to adapt to harsh environmental conditions. Experimental designs with more extreme conditions may help in the expression of the genetic potential of *Thellungiella* that would unravel how the extremophillic potential of *Thellulgiella* differ from the glycophytic strategies of the common model plant *Arabidopsis*.

6.0 RESPIRATORY PROPERTIES OF *Thellungiella* AND *Arabidopsis*

6.1 Introduction

Differential growth habits and adaptive strategies of plants may require altered respiratory inputs resulting in differential respiration in plant species under varied or changing conditions (Atkin and Day 1990; Wright et al. 2006). Usually fast growing species have higher rates of respiration (Poorter and Pothmann 1992; Gifford 2003). Respiration also shows a developmental dependence in that the respiration rate per unit of tissue weight decreases with increasing size and age of the plant organ (Poorter and Pothmann 1992; Villar et al. 1995; Armstrong et al. 2006a). Plant species growing at warmer, higher irradiance or lower-rainfall sites also have higher mean values of respiration (Wright et al. 2006). These observations imply that plant respiration is contingent upon ecological backgrounds, physiological status and growth habits of plants.

Changes in temperature have significant effects on the plant respiration. Respiration rate increases non-linearly with instantaneous changes in temperature (Atkin and Tjoelker 2003; Atkin et al. 2005a). The temperature sensitivity of respiration is more or less conserved across plant functional groups differing in maximum potential growth rate (Campbell et al. 2007). The relationship between respiration and temperature is a predictable physiological property of plants, with Q_{10} values (the factor by which respiration increases for a 10°C change in temperature) varying around 2 (Larigauderie and Koner 1995; Gifford 2003; Atkin et al. 2005a; 2005b). Respiration undergoes thermal acclimation, leading to a change in the temperature sensitivity of respiration, and thereby a change in the Q_{10} value. Plants acclimated to contrasting temperatures exhibit some degree of convergence in the rates of respiration at their respective temperatures. This condition is known as respiratory homeostasis (Tjolker et al. 1999; Atkin and Tjoelker 2003; Atkin et al. 2005a; 2005b). *Arabidopsis* leaves developed entirely at warm and cold temperatures display the property of respiratory homeostasis (Armstrong et al. 2006b).

An analysis of the photosynthetic properties of *Thellungiella* and *Arabidopsis* (Chapter 5) revealed several shared responses to environmental factors, as were seen in growth trend (Chapter 4) and freezing tolerance capacity (Chapter 3). However, the *Thellungiella* ecotypes set themselves apart from *Arabidopsis* in certain aspects when subjected to short- and long-term changes in temperature and irradiance (Chapter 5). However, these distinctions in photosynthetic properties between *Thellungiella* and *Arabidopsis* are still inadequate to explain the differential growth plasticity of the experimental taxa and specifically the arrested growth of Yukon under low irradiance (presented in Chapter 4). This led to the hypothesis that differential

dark respiration could be responsible for the differential growth plasticity and that the arrested growth of Yukon at low irradiance should be associated with high rates of dark respiration. Stress tolerance is energy-demanding property. Therefore, respiratory responses may be important for the extremophile *Thellungiella*. Characterization of respiratory properties of *Thellungiella* in comparison with the model plant *Arabidopsis* serves as the valuable benchmark for *Thellungiella* physiology. It is shown in this chapter that the dark respiration of experimental taxa exhibit differential interactions with environmental conditions. Comparative dark respiration of three experimental taxa at growth temperatures and the effect of short-term changes in temperature (measuring temperature) on dark respiration are presented in this chapter.

6.2 Materials and Methods

6.2.1 Plant Material and Growth Conditions

Seeds of the Yukon and Shandong ecotypes of *Thellungiella salsuginea* (Pall.) O.E. Schulz as well as *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia, Col-0) were germinated and maintained as described in Section 3.2.1 using the non-acclimating growth conditions described in Table 4.1 and cold acclimated as described in Section 5.2.1.

6.2.2 Respiration Measurements

Leaf respiration was measured polarographically *in vivo* by both the liquid- and gaseous-phases using a Clark-type oxygen electrode (Dilieu and Walker 1981). The electrode was calibrated to intended measurement temperatures that represented day-time growth temperatures of both the non-acclimating (20°C) and cold acclimating growth regimes (4.5°C). Measurements were also made reciprocally at 4.5°C for non-acclimated plants and 20°C for cold acclimated plants. Temperature was maintained by a refrigerated circulating water bath (Lauda-Königshofen, GmbH, Germany) in a Hansatech leaf-disc chamber (LD2/3; Hansatech Instruments Ltd, King's Lynn, Norfolk, UK) coupled with a computerized data acquisition system (Oxygraph Plus Version 1.31, Hansatech). The plant age during respiration measurements was 20 to 24 days for *Arabidopsis* and 25 to 30 days for Yukon and Shandong plants grown under non-acclimating growth regimes. Similarly for cold acclimated plants, the measurements were done after 20 to 24 days of cold acclimation for all taxa examined. After removal from the growth chamber, plants were placed in the dark (a black container covered with black cloth) for approximately 30 min at their respective growth temperatures or intended measurement temperatures. For cold-acclimated plants, temperature inside the container was maintained using ice and a chilled, damp cloth. Freshly harvested

fully expanded sample leaves were weighed in a balance prior to performing respiration measurements. Rates of O_2 uptake by the leaf samples were obtained as the slope of the linear regression curve from the straight linear portion of the respiration graph. The respiration rate was expressed on a FW basis as the quantity of oxygen consumption over time (nmol O_2 g FW $^{-1}$ s^{-1}).

6.2.2.1 Liquid-Phase

Liquid-phase measurements were determined in 1 mL of deionized water. The electrode was calibrated by using deoxygenated water (purged with N_2) to set a zero line and air saturated water to set a maximum gain value. The leaves from dark-incubated plants were cut into pieces of about 4 mm using a razor blade. This ensured that they could rotate freely inside the cuvette. Continuous mixing was provided by a stir bar inside the cuvette during measurement. The samples were allowed to equilibrate inside the cuvette for 5 min followed by recording of the uptake of O_2 by the leaf samples for 15 min.

6.2.2.2 Gaseous-Phase

For gaseous-phase measurements, the electrode was calibrated to a zero line using N_2 gas and to 21% O_2 by injecting 1 mL of room air at the measuring temperature. The leaves harvested from the dark-incubated plants were placed in the cuvette in such a way that the interior surface of the cuvette was covered. The samples were allowed to equilibrate inside the cuvette for 5 min followed by measuring the uptake of O_2 by the leaf samples for 15 min (Armstrong et al. 2006).

6.2.3 Calculation of Q_{10} Values

Measurement of respiration at reciprocal temperatures allowed for the calculation of the respiratory Q_{10}, the factor by which respiration increases for a 10°C change in temperature. The Q_{10} was calculated by using the following formula:

$$Q_{10} = (R/R_o)^{[10/(T-To)]}$$

Where R is the respiration rate at given temperature T, and R_o is the base rate of respiration at an arbitrarily set temperature T_o (Bruhn et al. 2008). In this study, the T and T_o were 20 and 4.5°C respectively.

6.2.4 Experimental Design and Data Analyses

Experiments and data analyses were performed using descriptive statistics, correlation and ANOVA techniques as described in Section 5.2.6.

6.3 Results

6.3.1 Dark Respiration in Altered Growth Regimes

Thellungiella ecotypes and *Arabidopsis* were grown under three different growth regimes and acclimated under respective cold temperature conditions. Measurements were taken at 20°C for non-acclimated plants and 4.5°C for cold acclimated plants; making the measurement temperatures close to the growth temperatures during the stage of measurements. The liquid phase respiration data were analyzed separately and then an overall analysis was also carried out in order to understand if the experimental taxa interact differentially with the growth regimes.

The overall analysis showed a significant difference ($P < 0.001$) in dark respiration between the non-acclimated and cold acclimated plants when measured at their respective growth temperatures. Experimental taxa differed significantly ($P = 0.001$) for dark respiration within the growth regimes. However, no significant difference ($P = 0.614$) was observed in dark respiration within a taxon across the growth regimes (Table 6.1). The two-way and three-way interactions between the acclimation status, growth regimes and the experimental taxa were significant ($P < 0.05$) suggesting that the experimental taxa had differential respiration across different environmental conditions.

Measured at the respective growth temperatures, the cold acclimated values of respiration were lower than those of the non-acclimated plants. However, these results are not surprising as the growth temperatures differed by approximately 15°C. Due to the temperature-dependent thermodynamic properties of enzymes, metabolic reactions are enhanced at higher temperature and therefore the respiratory energy demand is higher for fuelling the physiological processes.

In non-acclimated conditions, Yukon had generally higher rates of dark respiration than Shandong and *Arabidopsis* across all growth regimes. However, the values differed significantly only in the *Arabidopsis* regime, where Yukon growth was severely constrained and plants started dying out after four weeks of emergence. This result implied that the growth irradiance of 100 µmol photon m^{-2} s^{-1} in the *Arabidopsis* growth regime was the minimum to meet the metabolic energy demand of this ecotype and that Yukon has significantly higher light compensation point than that of Shandong and *Arabidopsis*. The taxonomic differences in respiration were also pronounced in cold acclimated conditions. However, barring the exceptions observed in Shandong regime, *Thellungiella* ecotypes displayed significantly lower dark respiration than *Arabidopsis* under cold acclimated conditions. In general, dark respiration was significantly

Table 6.1. Dark respiration in Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown under various growth regimes

Taxa	Growth regime			Growth regime		
	Yukon	Shandong	*Arabidopsis*	Yukon	Shandong	*Arabidopsis*
	Non-acclimated			Cold-acclimated		
Yukon	3.1 ± 0.58 $^{a\ A}$	2.9 ± 0.68 $^{a\ A}$	4.8 ± 0.51 $^{a\ A}$	1.0 ± 0.13 $^{b\ A}$	1.1 ± 0.12 $^{a\ AB}$	0.9 ± 0.05 $^{b\ B}$
Shandong	2.7 ± 0.29 $^{a\ A}$	2.7 ± 0.15 $^{a\ A}$	1.6 ± 0.28 $^{b\ B}$	0.9 ± 0.03 $^{b\ AB}$	0.9 ± 0.09 $^{a\ A}$	0.7 ± 0.06 $^{b\ B}$
Arabidopsis	2.8 ± 0.57 $^{a\ A}$	2.6 ± 0.22 $^{a\ A}$	2.8 ± 0.49 $^{b\ A}$	2.1 ± 0.02 $^{a\ A}$	1.2 ± 0.20 $^{a\ B}$	1.3 ± 0.14 $^{a\ B}$

Respiration was measured in the liquid-phase and values represent means ± SE (n = 3 to 6)
The data were analyzed using ANOVA and means separated by Fisher's individual error rate method
Values followed by different small letters differ significantly within the columns, while different capital letters denote the significant difference along the row within an acclimation state
ANOVA, anaysis of variance; SE, standard error

lower in the *Arabidopsis* growth regime that mainly differed in irradiance from other two growth regimes (Table 6.1).

6.3.2 Dark Respiration in the Yukon Growth Regime

Leaf dark respiration of Shandong, Yukon and *Arabidopsis* was measured in the gas-phase at two different temperatures that represented the non-acclimated and cold acclimated temperatures in the Yukon growth regime. These two measurement temperatures were 20 and 4.5°C respectively. Table 6.2 presents the summary results of the gas-phase measurements of dark respiration.

Overall analysis of the sources of variation showed that respiration rates were significantly affected by the acclimation status ($P < 0.001$), taxa ($P < 0.001$) and measurement temperature ($P < 0.001$). The taxa displayed significant interaction ($P = 0.005$) with the measuring temperature. The interaction between the acclimation status and the measurement temperature was also highly significant ($P < 0.001$).

The data presented in Table 6.2 shows that respiration measured at low temperature had significantly lower values than that of the warm temperature. When measured at warm temperature, cold acclimated plants had significantly higher respiration rates than those of non-acclimated plants. This may be due to the abundance of readily available carbohydrate as the respiratory substrate in the cold acclimated plants. However, when measured at low temperature, respiration rates of non-acclimated and cold acclimated plants appeared similar to each other in all three taxa.

Separate analysis of respiration rates of non-acclimated plants showed significant differences between the experimental taxa ($P = 0.002$) and measurement temperatures ($P < 0.001$). The interaction between the taxa and the measurement temperature was non-significant suggesting that the taxa responded to the measurement temperatures in a similar pattern. The analysis within non-acclimated conditions measured at respective growth temperature showed significant differences in the rates of respiration ($P = 0.023$) between the taxa. The respiration rates of Yukon were about 37% and 22% higher than that of Shandong and *Arabidopsis* respectively (Table 6.2). The differences between Yukon and Shandong were statistically highly significant, while *Arabidopsis* remained statistically at par with both contrasting ecotypes of *Thellungiella*. In contrast, at the low measurement temperature the non-acclimated plants of the experimental taxa did not differ significantly for leaf respiration ($P = 0.065$). Under the cold acclimated conditions, there were significant differences ($P < 0.001$) in leaf respiration between the experimental taxa and between the measurement temperatures, with the significant interactions ($P = 0.002$)

Table 6.2. Dark respiration and respiratory Q_{10} values in Yukon and Shandong ecotypes of *Thellungiella* and *Arabidopsis* grown in the Yukon growth regime

Taxa	Dark respiration (nmol O_2 g FW $^{-1}$ s^{-1})					
	Non-acclimated			Cold-acclimated		
	Measuring temperature		Q_{10}	Measuring temperature		Q_{10}
	20°C	4.5°C		20°C	4.5°C	
Arabidopsis	7.95 ± 0.61[ab]	3.76 ± 0.71[a]	1.70 ± 0.22	12.27 ± 0.89 [a]	5.39 ± 0.48 [a]	1.62 ± 0.10
Shandong	7.05 ± 0.44[b]	4.13 ± 0.29[a]	1.37± 0.07	9.05 ± 0.43 [b]	4.77 ± 0.39 [a]	1.46 ± 0.06
Yukon	9.68 ± 0.73[a]	5.82 ± 0.71[a]	1.38 ± 0.09	14.1 ± 0.86 [a]	5.08 ± 0.40 [a]	1.82 ± 0.13

Respiration was measured in the gas-phase and values represent means ± SE (*n* = 3 to 6)
The data were analyzed using ANOVA and means separated by Fisher's individual error rate method
Values followed by different letters within the columns differ significantly
ANOVA, anaysis of variance; SE, standard error

between experimental taxa and temperature. Within the warm measurement temperature, Yukon exhibited about 55% and 15% higher rates of leaf respiration than that of Shandong and *Arabidopsis* respectively (Table 6.2). In statistical terms, the warm measured respiration rates of cold acclimated Shandong were significantly (P = 0.001) lower than that of Yukon and *Arabidopsis*, while the later stood statistically at par. However, within the low measurement temperature, the taxonomic differences were non-significant.

The higher dark respiration rates of Yukon were also associated with a lower ULR, a measure of net assimilation rate (Causton 1994; Figure 6.1). There was negative correlation between the ULR and dark respiration (r = -0.703), but the extent of correlations differed between the taxa. The correlation coefficients of Shandong and *Arabidopsis* were much higher (r = -0.902 and -0.978 respectively) than that of Yukon (r = - 0.703).

6.3.3 Respiratory Q_{10} of *Thellungiella* and *Arabidopsis* in the Yukon Growth Regime

The respiratory Q_{10} was calculated based on the respiration rates of the measurement temperatures (20°C and 4.5°C) of both non-acclimated and cold acclimated plants grown under the Yukon growth regimes. The analysis showed that there was no significant effect (P > 0.12) of genetic as well as environmental factors on the Q_{10} values of *Thellungiella* and *Arabidopsis* (Table 6.2). This suggests that the respiratory Q_{10} is fairly conserved trait between these experimental taxa.

6.4 Discussion

6.4.1 The Yukon Ecotype has the Highest Rate of Dark Respiration

A generalized observation from both liquid- and gas-phase measurement is that the dark respiration for non-acclimated plants of Yukon is higher than that of Shandong and *Arabidopsis*, while the later exhibited more or less similar trends (Table 6.1 and 6.2). These results correspond with growth phenotypes of the experimental taxa in different growth regimes. The stagnant growth of Yukon in *Arabidopsis* growth regime (low irradiance of 100 µmol photons $m^{-2}s^{-1}$) can be attributed to the higher dark respiration that would result in the lack of net assimilation for maintaining the growth of plants. The higher respiration rates of Yukon were associated with a lower ULR (Figure 6.1) and a correlation analysis implicates differential interactions between the photosynthetic and respiratory metabolism of Yukon and the

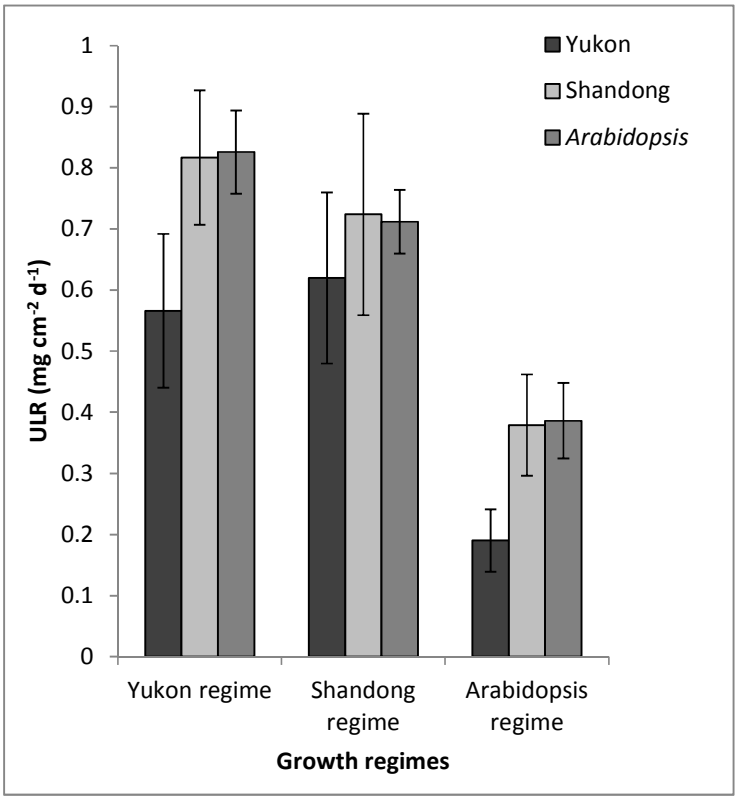

Figure 6.1. Effect of growth regime on unit leaf rates. *Thellungiella* ecotypes and *Arabidopsis* were developed under the growth regimes indicated. Yukon ecotype (■); Shandong ecotype (■); *Arabidopsis* (■). Values represent means ± SE (n = 3) from 3 stages of measurements

other experimental taxa. The high rate of respiration of Yukon and its inability to grow and complete its life cycle under low irradiance conditions suggests that this ecotype possesses a less efficient carbon economy than that of Shandong and *Arabidopsis*. This may be associated with the adaptive attribute of plants that evolve in stress-prone environments which require a high investment of energy through high maintenance respiration for the constitutive deployment of stress-response mechanisms (Chapin III et al. 1993; Block et al. 2009). There is also an implication for higher photorespiration in *Thellungiella* as evidenced by the higher expression of photorespiration related enzymes, GGT1 and glycine cleavage system H protein 2 that partly explains the lower growth rate in *Thellungiella* compared with *Arabidopsis* (http://www.technology-articles.org/Botany/Proteomic-Analysis-of-Thellungiella-Salsuginea.html). Thellungiella may have a higher level photorespiration, which limited the growth rate. In *Arabidopsis*, a variety of alternative respiratory pathways affecting metabolic efficiency have been identified and those pathways are usually up-regulated by altered environmental conditions (Escobar et al. 2006). There are virtually no studies carried out examining the respiratory properties of *Thellungiella* (Amtmann 2009).

6.4.2 Respiratory Q_{10} is Conserved in the Experimental Taxa

In this study, the respiratory Q_{10} was calculated from the dark respiration values obtained from measurement temperatures of 20°C and 4.5°C of both non-acclimated and cold acclimated plants. The mean Q_{10} values of Yukon, Shandong and *Arabidopsis* varied from 1.37 ± 0.07 to 1.82 ± 0.13 with no significant differences in values between the experimental taxa (Table 6.2). This suggests that the respiratory Q_{10} is a fairly conserved trait between these experimental taxa. The Q_{10} values obtained from this study are very close to a generalized Q_{10} value of 2 established by previous studies. The recorded Q_{10} values for leaves vary from 1.4 to 4.2 and for roots from 1.1 to 4.6 (Atkin et al. 2005b). It is now well accepted that the temperature response curve of respiration is non-linear and different ranges of temperature selected for Q_{10} calculations may result in discrepancies of the resultant values. Moreover, differences in controlled experimental variables such as plant nutrient and water regimes, season and other biotic and abiotic factors affecting plant respiration may result in wider inter- and intra-specific variation in the Q_{10} values across different experiments (Atkin et al. 2005b). In fact, the Q_{10} values of several plant species derived from common ranges of temperature have been found to converge to a value close to 2. For example, in 65 species, the Q_{10} value of 2.50 ± 0.11 was derived from the measurement temperature range with mid-point of 15°C (Tjoelker et al. 2001). In an earlier study with 34 species from temperate and arctic regions, the Q_{10} values derived from the temperature range of 10°C and 20°C remained around 2.45

± 0.17. Similarly, a compilation of Q_{10} values of 125 species from published literature came up with a mean of 2.3 with most values lying between 2.0 and 2.5 (Larigauderie and Koner 1995). In the high mountain plant species *Ranunculus glacialis*, Nogués et al. (2006) observed a high rate of dark respiration with a Q_{10} of about 2 in the temperature range of 10°C and 18°C. These data suggest that the respiratory Q_{10} in a given range of measurement temperature and similar resource regimes is comparable among plant species. A recent finding suggests a global convergence in the temperature sensitivity of respiration at the ecosystem level with Q_{10} values confined around 1.4 ± 1 across biomes without any effect of the mean annual temperatures (Mahecha et al. 2010).

6.4.3 Thermal Acclimation of Dark Respiration between Experimental Taxa

Like several physiological processes, plant respiration also acclimates to altered growth temperatures. The thermal acclimation of respiration may result in a change in the temperature sensitivity of respiration. In this study, when measured at low temperature (4.5°C), the non-acclimated and cold acclimated plants of all three experimental taxa exhibited convergence of respiration rates to similar values that were drastically lower than those measured at warm temperatures (20°C). On the other hand, when measured at warm temperatures, the cold acclimated plants of all experimental taxa showed higher rates of respiration than those of non-acclimated plants. Compared to non-acclimated counterparts, the dark respiration of cold acclimated plants measured at warm temperature was higher by 54%, 45% and 28% in *Arabidopsis*, Yukon and Shandong respectively.

Earlier studies have shown that plant species exhibit different types of respiratory responses due to cold acclimation. In some instances, cold acclimation results in a change in slope of the temperature response curve without a change in the intercept. This means that cold acclimated plants exhibit an increase in the rate of respiration only at higher measurement temperatures. The experimental taxa in this study displayed the above type of acclimation with no significant difference in the respiration values of cold acclimated and non-acclimated plants at low measurement temperature (Table 6.2). In some other instances, cold acclimation leads to the change in the intercept and an entire shift in the respiratory temperature response curve. This means that there is increase in the rate of respiration over a wide range of temperature due to cold acclimation (Atkin and Tjoelker 2003; Atkin et al. 2005a; 2005b). Armstrong et al. (2006b) reported that *Arabidopsis* leaves developed entirely at warm and cold temperatures displayed the property of respiratory homeostasis (some degree of convergence in the rates of respiration at their

respective growth temperatures). The degree of respiratory homeostasis appeared to be similar in all three of the experimental taxa (Table 6.2).

6.5 Conclusions

The findings of this chapter support the hypothesis that the differential growth rates observed in Yukon, Shandong and *Arabidopsis* are attributable to their differential dark respiration rates. A distinctive feature exhibited by the Yukon ecotype was its significantly higher rate of dark respiration and inability to maintain growth and development under low irradiance conditions. The stagnant growth of Yukon in the *Arabidopsis* growth regime corresponded well with its higher rate of respiration compared to other experimental taxa. Despite the differential dark respiration, all experimental taxa exhibited similar respiratory Q_{10} values and a degree of respiratory homeostasis. It is an intriguing area for further studies as to what underlying physiological processes drive higher rates of dark respiration in the Yukon ecotype of *Thellungiella*.

7.0 GENERAL DISCUSSION

7.1 Comparative Physiology of *Thellungiella* and *Arabidopsis*

This study involved a physiological comparison of two *Thellungiella* ecotypes, namely Yukon and Shandong, with reference to most widely used model plant *Arabidopsis*, Columbia ecotype. These experimental taxa are herein referred to as Yukon, Shandong and *Arabidopsis* for the simplicity. *Arabidopsis* is a typical glycophytic plant, while Shandong and Yukon are considered extremophillic, thriving in the conditions of multiple stresses. In the context of the emergence of *Thellungiella* ecotypes as alternative model systems for the examination of stress tolerance, this study was tempted by two research questions:

i) what are the shared and differential physiological responses of *Thellungiella* and *Arabidopsis* in terms of growth, photosynthesis and freezing tolerance?

ii) does *Thellungiella* promise to offer a comparative advantage to *Arabidopsis* for the elucidation of freezing tolerance and photosynthetic acclimation in plants?

In attempt to address these research questions, the study covered the aspects of growth, photosynthesis, respiration and cold tolerance under different growth regimes varying in temperature, irradiance and photoperiod. Both the non-acclimating and cold acclimating growth regimes used in this study were reference conditions frequently utilized in previous studies and lie within the adaptive range of *Arabidopsis*.

Within the environmental conditions represented in the experimental design, Yukon, Shandong and *Arabidopsis* displayed identical directionality of physiological responses to the environment. For example, all ecotypes showed enhanced growth rate and phenological development with an increase in daily photon irradiance (DPI, an expression combining both irradiance and duration) and cumulative daily average temperature above the threshold temperature of 4°C (modified growing degree days – MGDD). Both *Thellungiella* and *Arabidopsis* germinated and successfully completed their life cycle under continuous cold environments with a temperature as low as 4°C. These observations corroborate the earlier studies on *Arabidopsis* (Meng et al. 2008) and the Yukon ecotype of *Thellungiella* (Griffith et al. 2007). In all three experimental taxa, a high correlation was observed between various absolute growth variables such as dry matter content, leaf area, number of leaves and rosette radius, thereby permitting the use of non-invasive sampling for the quantitative estimation of the relative size of plants. The photosynthetic competence of PSII also followed a similar directionality of response as that of growth. In all taxa, cold acclimation culminated in the modulation of photosynthetic and respiratory indicators in the same direction, invariably

resulting in enhanced tolerance to freezing. Likewise, the instantaneous exposure of non-acclimated and cold acclimated plants to reciprocal temperatures engendered a common manifestation of a cold-induced down-regulation and thermal up-regulation of photosynthesis and respiration. The respiratory Q_{10} was a conserved feature in these taxa with values lying around 1.5, which is considered representative of many plant species. Yukon, Shandong and *Arabidopsis* grown under identical non-acclimating conditions and/or subjected to one week of cold acclimation displayed a similar extent of freezing tolerance. All ecotypes showed a convergence of their floral transition when grown under low temperatures (4 to 8ºC) and long-day (16 h photoperiod) conditions with moderate light intensities (250 to 350 µmol photons m^{-2} s^{-1}).

Amidst the similarity of environmental responses, Yukon, Shandong and *Arabidopsis* displayed a few physiological contrasts and some quantitative differences for several response variables. *Arabidopsis* invariably showed faster growth rate and phenological development than both ecotypes of *Thellungiella*. Yukon showed a significantly higher sensitivity to growth irradiance, manifested by its inability to meet its physiological energy requirement at low light levels (100 µmol photons m^{-2} s^{-1}), while Shandong shared some commonality with *Arabidopsis* by having more stable responses to growth irradiance. In all growth conditions, the reproductive transition occurred unabatedly in *Arabidopsis*, with a hastening effect of increased photoperiod and temperature. In Yukon and Shandong, on the other hand, either vernalization or ecotype-specific irradiance seemed to be required to trigger the reproductive transition. Two weeks of vernalization triggered the reproductive transition in both Yukon and Shandong. Yukon required higher light intensities (at least 350 µmol photons m^{-2} s^{-1}) for floral initiation, while Shandong flowered without vernalization under low irradiance conditions (100 µmol photons m^{-2} s^{-1}). Similar light-dependent flowering responses were also reported in *Crowea exalata* (King et al., 2008). Yukon, Shandong and *Arabidopsis* displayed differential modulation of photosynthetic pigmentation and P_{700} oxidation in response to short- and long-term variability in the environmental factors. Like the stability of growth, Shandong showed more resilient photosynthetic and respiratory properties, compared to both Yukon and *Arabidopsis*. From the same level of basal tolerance to freezing, both ecotypes of *Thellungiella* displayed substantially higher acclimative gains in freezing tolerance than *Arabidopsis*. Moreover, a combination of 4-days of drought followed by one week of cold acclimation had a synergistic effect on freezing tolerance, while no such effect was observed in *Arabidopsis*.

The above comparative physiological synopsis substantiates that Yukon, Shandong and *Arabidopsis* are the subject of distinctive scientific curiosities. In the perspective of adaptive research, the physiological properties of Yukon, Shandong and *Arabidopsis* possess complementary practical relevance.

Each of the experimental taxa can be used as a model system for environment-specific ecological adaptation that may pertain to crop farming in stress-prone environments, phytoremediation of disturbed ecosystems and habitat management for biodiversity conservation or simply the extension of frontiers of experimental biology. The comparative growth, freezing tolerance, photosynthesis and respiratory physiology of these taxa is presented in the foregoing discussions of Chapter 3 though to Chapter 6. Hereunder follows the summative view on the physiological responses of the individual taxa in the light of their comparative advantage in experimental biology.

7.2 *Arabidopsis* – A Versatile Model Plant for Experimental Efficiency

The ability of *Arabidopsis* to germinate, grow and complete its lifecycle in the wide range of temperature is very conducive for experimental efficiency with features such as a short lifecycle, amenable growth form, simple genetics and transformability already established (Boyes et al. 2001; Suh et al. 2003; Samis et al. 2008; Koornneef and Meinke 2010). In our experimental conditions, *Arabidopsis* growth and phenological development responded positively to the temperature, growth irradiance and extension of photoperiod from 16 h to 21 h. Under warm-grown conditions with 22º/10ºC, 250 µmol photons m^{-2} s^{-1} and 21 h photoperiod, *Arabidopsis* seedlings emerged as early as 3 days after planting and attained visually a full rosette and bolting stage within 3 weeks, leading to the termination of its inherently indeterminate reproductive phenology within two months after planting (Table 4.1 for example from the Yukon growth regime). Thus, the earliest seed-to-seed cycle was complete within 6 weeks of planting. Earlier studies reported similar observations on *Arabidopsis* under long-day conditions (Koornneef et al. 1991; Cookson et al. 2007). On the other hand, a four-fold decrease in temperature from 20 to 5ºC keeping growth irradianjce at 100 µmol photons m^{-2} s^{-1} and photoperiod at 16 h resulted in the spanning of its indeterminate growth for over one year with floral stock height of nearly one meter, a 6 times extension of plant life and three times more height than that of most growth enhancing conditions (Yukon growth regime). There was no vernalization requirement for the reproductive transition in *Arabidopsis* in any of the growth conditions tested (4 to 22ºC, 100 to 450 µmol photons m^{-2} s^{-1} and 16 to 21 h photoperiod). *Arabidopsis* displayed comparable photosynthetic and photoprotective competence of PSII to that of Yukon and Shandong in response to cold acclimation, short-term exposure to cold (4ºC for 20 min to 4 h) and instantaneous increases in PPFD from 55 to 1790 µmol photons m^{-2} s^{-1}. Interestingly, *Arabidopsis* stood at par with *Thellungiella* counterparts for basal freezing tolerance and a short-term (1 week) cold acclimative gain in freezing tolerance. This shows that when grown under identical conditions, the physiological plasticity of

Arabidopsis is comparable to that of its extremophillic counterparts Yukon and Shandong. Hence, *Arabidopsis* holds its supremacy as a versatile model system for developmental and physiological studies.

7.3 Shandong – A Model Plant for Studies of Physiological Resiliency

The major contrast between Shandong and *Arabidopsis* lied in the aspect of growth phenology, PSI homeostasis and acclimative potential to tolerate freezing. Shandong exhibited a facultative vernalization requirement in that in warm-grown plants under moderate light (250 µmol photons m^{-2} s^{-1}) and long-day (16 to 21 h) conditions required two weeks of vernalization for the reproductive transition, while those grown at lower irradiance (100 to 150 µmol photons m^{-2} s^{-1}) under similar warm and long-day conditions and those grown at cold-temperature (4 to 8ºC) flowered spontaneously within 3 months of planting. This observation contrasts with some earlier studies in that Shandong was reported to have an obligate vernalization requirement of about 3 weeks in order to flower (Bressan et al. 2001), while without vernalization, flowering occurred after 10 to 12 months of plant emergence (Inan et al. 2004). The contrasting observations between present and earlier studies suggest that our understanding of the growth and reproductive behavior of Shandong is still lacking. It can be inferred from our results that appropriate light and temperature regimes can replace the vernalization requirement for Shandong. This phenological peculiarity of Shandong makes it an interesting model for developmental biology of plants. Further studies are needed to analyze its photoperiodicity and other environmental specificities for flowering.

Compared to *Arabidopsis* and Yukon, this ecotype exhibited consistently higher and more stable patterns of P_{700} oxidation in response to short- and long-term environmental variables. Under similar conditions of excitation pressure, Shandong exhibited quantitatively superior energy partitioning in favour of PSII photochemistry than that of Yukon and *Arabidopsis*. Based on a previous study (Stepien and Johnson 2009), the increased electron transport rate through PSII in Shandong may be implicated to chlororespiration. Stepien and Johnson (2009) observed that better tolerance of *Thellungiella* Shandong to salinity compared to that of *Arabidopsis* was associated with the up-regulation of the plastid terminal oxidase (PTOX) and chlororespiration, accounting for up to 30% of the total electron transport through PSII (ETR_{PSII}), while there was no such up-regulation in *Arabidopsis*.

While acclimative gains in freezing tolerance of *Arabidopsis* exhausted beyond 2 weeks of cold-acclimation, Shandong continued gaining freezing tolerance with further increases in the duration of cold acclimation. These observations corresponded with the results of a recent proteomic study of *Thellungiella* rosette leaves where an increase in low temperature exposure time from 2 h through 5 to 24 days

augmented the abundance of dozens of cold-responsive proteins (Gao et al. 2009). The increase in freezing tolerance with the increasing acclimation time may be attributed to attainment of a better homeostatic state with the development of multiple protection mechanisms at molecular, histological or morphological levels. A more striking difference between Shandong and *Arabidopsis* is the synergistic acclimative gain through the exposure to combination of 4-days drought followed by 1-week of cold acclimation. In Shandong, the acclimative combination of drought and cold resulted in approximately a 50% increase in the degree of freezing tolerance from what is maximally attainable through cold acclimation alone. There was no such synergistic gain in *Arabidopsis*. The maximal acclimative gain in freezing tolerance was higher (-17.8ºC) in Shandong, compared to that in *Arabidopsis*. Hence, Shandong being endowed with the feature of greater growth stability, greater photosynthetic homeostasis and stress-tolerance potential, can become the model system of choice for diverse physiological specialties.

7.4 Yukon – An Experimental Curiosity for Environmental Specificity

Highly plastic growth responses to growth irradiance and its inherently higher respiratory flux was the distinctive feature of Yukon that set itself aside from its counterparts Shandong and *Arabidopsis*. High rates of dark respiration and its inability to grow and complete its life cycle at low irradiance (100 µmol photons m^{-2} s^{-1}) suggests a less efficient carbon economy than that of Shandong and *Arabidopsis*. This may be associated with the adaptive attribute of plants that have evolved through stress-prone environments requiring a high investment of energy for the constitutive deployment of stress-response mechanisms. However, this reasoning is not supported by the fact that Yukon, Shandong and *Arabidopsis* had similar levels of basal freezing tolerance and had identical inter-ecotypic acclimative potential until one week of cold acclimation. Furthermore, remarkably exceptional increases in freezing tolerance of Yukon with the increase in the duration of cold acclimation until 3 weeks suggests a regulated, inducible pattern of freezing tolerance rather than one that is constitutive nature. Yukon attained a substantially greater degree of freezing tolerance than that of Shandong and *Arabidopsis* through cold acclimation for over two weeks. Like in Shandong, the acclimative combination of drought and cold treatment extended its freezing tolerance to -25ºC which was beyond the maximal value (-22ºC) attained through 3-weeks of cold acclimation alone. The maximal acclimative gain in freezing tolerance was similar to that of Shandong and drastically higher than that of *Arabidopsis*.

Another peculiarity of Yukon, like that of Shandong, was its facultative vernalization requirement for reproductive transition. Two-weeks of vernalization was adequate for triggering the reproductive transition

of warm-grown plants under moderate light (250 µmol photons m^{-2} s^{-1}) and long-day (16 to 21 h) conditions. This ecotype failed to attain reproductive competence at lower irradiance (100 µmol photons m^{-2} s^{-1}) resulting in the premature degeneration of the plants. Spontaneous flowering without vernalization occurred in around two months under warm, long-day and higher PPFD (350 to 450 µmol photons m^{-2} s^{-1}) conditions and under continuously cold-grown conditions (4 to 8ºC) with moderate to higher irradiances. Yukon stood intermediate between Shandong and *Arabidopsis* in photosynthetic competence. Exceptionally, Yukon contrasted with Shandong with respect to cold acclimative augmentation of P_{700} oxidation, in that Yukon had approximately a 67% increase and Shandong had a 9% decrease in the values of P_{700} oxidation due to cold acclimation. However, it is notable that Yukon had the lowest oxidizable P_{700} in the *Arabidopsis* growth regime that presumably barely met the light compensation point of this ecotype resulting in stagnant growth and eventual collapse of the plants after 4 weeks of germination. This shows an interesting relationship between P_{700} oxidation and plant growth in Yukon ecotype, supported by an earlier study examining oxidizable P_{700} and CO_2 assimilation (Harbinson and Hedley 1993). The peculiar growth, phenology and respiratory features, and exceptionally high degree of freezing tolerance make this ecotype an experimental choice for unique physiological specificities.

7.5 Conclusions and Recommendations for Further Research

The contribution of *Arabidopsis* in enhancing the efficiency of plant biological research can hardly be over-emphasized. However, the glycophytic nature of *Arabidopsis* constrains both scientific curiosity and practical relevance of the studies that go beyond the adaptive limits of *Arabidopsis*. Identification of extremophillic plant model systems from close relatives of *Arabidopsis* can overcome the experimental constraints associated with *Arabidopsis*, while capitalizing on the existing wealth of knowledge and resources available. This study examined comparative phenotypic responses of Yukon, Shandong and *Arabidopsis* under different growth regimes. The study unraveled several similarities and contrasts between these experimental taxa in the aspects of growth and phenological development, photosynthesis, respiration and freezing tolerance. The generic responses to growth environment, cold acclimation, short-term exposure to contrasting temperatures and increasing irradiance or photoinhibitory treatments were very similar between these taxa. Amidst these similarities, each of the taxa exhibited physiological distinctions with context-specific advantages for their use as complementary model systems for developmental and stress physiology studies. *Arabidopsis* held its supremacy to be a model system for its shorter and plastic life cycle, relative stability of growth across diverse growth conditions, PSII

photosynthetic competence and constitutive tolerance to freezing comparable to that of the extremophillic *Thellungiella* counterparts. On the other hand, both ecotypes of *Thellungiella* exhibited a distinctive performance of PSI, a substantially greater degree of stress-inducible tolerance to freezing and contrasting responses to environmental stimuli for reproductive transitions. The inter-ecotypic differences between Shandong and Yukon include contrasting effects of cold acclimation on PSI activity, flowering responses in different environments and differential plasticity to growth irradiance. The inter-specific and inter-ecotypic physiological contrasts between Yukon, Shandong and *Arabidopsis*, amidst common generic responses to environmental factors make them complementary model systems with potential to expand the frontiers of physiological studies.

Besides addressing the research question as to whether the physiological responses of *Thellungiella* and *Arabidopsis* reflect their contrasting ecological background thereby justifying the use of *Thellungiella* as alternative model systems for developmental and physiological studies, the study engendered number of curiosities to be addressed through further research.

All experimental taxa responded with enhanced growth to the increase in daily photon irradiance (DPI) from 5.75 mol photons m^{-2} d^{-1} (*Arabidopsis* growth regime) through 14.4 mol photons m^{-2} d^{-1} (Shandong growth regime) to 18.9 to mol photons m^{-2} d^{-1} (Yukon growth regime). However, the Yukon ecotype showed higher growth plasticity to DPI and lower growth plasticity to MGDD than that of Shandong and *Arabidopsis*. With a 1% increase in the DPI, there was a 2.3% increase in Yukon dry weight, which is drastically higher than that of Shandong and *Arabidopsis*. In the *Arabidopsis* growth regime, Yukon growth was severely arrested with a progressive decline and death of plants beyond 4 weeks of age. These results implicated that Yukon has higher light compensation point due to the requirement of high energy to maintain its innate stress tolerance mechanism. Further studies with photosynthetic and respiratory gas-exchange would confirm this proposition.

Both Yukon and Shandong ecotypes flowered without vernalization in 2-3 months under specific growth conditions. It seems that Yukon and Shandong respond differentially to the floral induction stimuli emanated from the complex interplay of irradiance, daily photoperiod and ambient temperature. Yukon grown at 350 to 450 µmol photons m^{-2} s^{-1} PPFD, 20^{o}C daily temperature and 16 h photoperiod flowered in about 2 months, while Shandong grown at 100 µmol photons m^{-2} s^{-1} with the same temperature and photoperiod flowered in about 3 months. Upon cold treatment at 4^{o}C for 15 days (between 35 and 50 days after sowing), the *Thellungiella* plants grown in typical *Arabidopsis*, Yukon and Shandong regimes flowered after two months. It was also found that if grown under continuously low temperatures with moderate

irradiance, *Thellungiella* started flowering around two months after sowing. It can be inferred from these results that appropriate light and temperature regimes can replace the vernalization requirement for *Thellungiella* ecotypes. Further studies are needed to analyze their photoperiodicity and other environmental specificities for flowering.

Thellungiella ecotypes showed slower phenological development than *Arabidopsis* across wide range of growth regimes characterized by different combination of temperatures and irradiances. However, the ecotypic differences in growth rate and phenology significantly narrowed in continuous low temperature grown conditions. In an earlier study, *Arabidopsis* leaf initiation and expansion rates were found linearly related to the temperatures in the range of 6 to 26°C, with the common x-intercept of 3°C. This suggests that 3°C is the threshold temperature for the initiation and development of *Arabidopsis* leaves (Granier et al. 2002). It would be interesting to carry out further comparative studies on *Arabidopsis* and *Thellungiella* to understand the relationship between the temperature, light variables and developmental transitions.

With a common level of basal freezing tolerance, there was an increasing trend in freezing tolerance with the increase in duration of cold acclimation until two weeks in *Arabidopsis* and three weeks in *Thellungiella*. These observations corresponded with the results of recent proteomic study in *Thellungiella* rosette where an increase in cold exposure time from 2 h through 5 to 24 d augmented the abundance of dozens of cold-responsive proteins (Gao et al. 2009). It can be hypothesized that the increase in freezing tolerance with the increasing acclimation time may be due to the development of multiple protection mechanisms at molecular, histological or morphological level. Combinations of drought and cold acclimation resulted in a further increase in the freezing tolerance of *Thellungiella*, but not in *Arabidopsis*. Further studies combining freezing tolerance assays and transcriptomic/proteomic studies are needed to unravel the underlying mechanism of the higher degree of inducible freezing tolerance in *Thellungiella* than that of *Arabidopsis*. A recent study showed that the freezing tolerance of *Arabidopsis* increased from about -6°C (LT_{50}) in non-acclimated plants to -13°C after 7 days of cold acclimation at 3°C. Sub-zero acclimation of cold acclimated plants with the exposure at -3°C for 3 days led to the further enhancement of freezing tolerance of *Arabidopsis* plants to -16°C (Livingston III et al. 2007). As a cold-adapted extremophile, *Thellungiella* may have even a more effective mechanism of sub-zero acclimation. Comparative studies of Yukon and Shandong on the aspect of sub-zero acclimation and its physiological basis are an interesting area for further study.

Chlorophyll fluorescence imaging can be advantageously applied as a non-invasive freezing tolerance assay for qualitative categorization of the plant responses into survival, uncertainty and lethal

states. It was realized through the experimentation that there is a need for refinement of the imaging protocol, especially in the aspect of irradiance level during the freezing and thawing process and the post-freezing waiting time before imaging measurements. With a standardized protocol, the qualitative classification approach may have better convenience and practicality in the mass screening of crop plants for freezing tolerance. Further studies on various plants are needed to establish the utility of this approach in plant screening for freezing tolerance.

Considering the extremophillic adaptation of *Thellungiella* in natural habitats that contrast with glycophytic adaptation of *Arabidopsis*, it was anticipated that *Thellungiella* possess better resiliency of PSII performance, especially under low temperature conditions. However, only Shandong displayed minor quantitative difference from *Arabidopsis*, while Yukon appeared fairly similar to *Arabidopsis*. It may be because of the fact that the treatments imposed in the experiments were within the adaptive range of *Arabidopsis*. Therefore, further comparative studies between *Thellungiella* and *Arabidopsis* with more extreme environmental conditions would be tempting to unravel the divergence of glycophytic and extremophillic photosynthetic adaptation.

In Shandong, P_{700} oxidation showed a growth irradiance-dependent acclimation pattern. There was a marked increase in oxidizable P_{700} as a result of acclimation in the *Arabidopsis* growth regime (low irradiance), but no acclimatory response in the Yukon and Shandong growth regimes (higher irradiance). Based on a recent study (Stepien and Johnson 2009), this is presumably due to enhancement of the PTOX activity of Shandong at higher irradiance, rendering acclimatory adjustment in PSI unnecessary in the given environmental conditions. This argument can be supported by the fact that salinity treatment triggered significant up-regulation of PTOX with concomitant increase in ETR_{PSII} in Shandong, while there was no such up-regulation of PTOX and ETR_{PSII} was reduced in *Arabidopsis* (Stepien and Johnson 2009). In our study, higher irradiance could have triggered such a response in Shandong. Presumably, the irradiance was not high enough for triggering PTOX or other alternative mechanisms in Yukon, as this ecotype had more sensitive growth plasticity to irradiance suggesting a higher irradiance requirement for its optimal growth than that of Shandong and *Arabidopsis*. Further studies are needed to test this hypothesis. In general, Shandong showed a trend of increasing e^-/P_{700} with increasing irradiance and due to cold acclimation. In Shandong, there is some correspondence between the e^-/P_{700} and ETR_{PSII} that explains partly what determines the e^-/P_{700} size. However, Yukon did not display any definite trend of e^-/P_{700} in response to environmental variables, while *Arabidopsis* responded to cold acclimation with an increase in e^-/P_{700}, without any definite trend across the growth regimes. Moreover, Yukon and *Arabidopsis* plants that

had apparently perturbed phenotypes in two contrasting growth regimes and acclimation status showed an exceptional escalation of e^-/P_{700} in response to two contrasting measurement temperatures. In Yukon, those observations were associated with warm temperature measurements of the plants from the non-acclimated *Arabidopsis* growth regime, where plant growth was arrested due presumably to deficit of irradiance to meet the metabolic demand of the plants. In *Arabidopsis*, on the other hand, the exceptional results were associated with low temperature measurement of cold acclimated plants from the Yukon growth regime, where plant phenotypes were chlorotic in nature. In fact, cold acclimated *Arabidopsis* had significantly lower chlorophyll content in the Yukon growth regime compared to other growth regimes. These results demonstrate that there is a complex interaction between the genotypes and environmental variables that determine the relative predominance of electron flux in the intersystem pool. Further studies are needed on the aspect of alternative electron sinks under different environmental conditions to unravel this mystery.

The unique physiological properties of the *Thellungiella* ecotypes Yukon and Shandong that contrast each other and from the model plant *Arabidopsis*, hold good promise for understanding extremophillic physiology and the effects of global climate change on the adaptive responses of plants in alpine and sub-arctic habitats.

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Appendix A: Statistical Output for Chapter 3

Arabidopsis: 4-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     403  (Event)
          dead     304
          Total    707

Frequency: Frequency

Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -211.070    20.7842  -10.16  0.000
Temp       0.810069  0.0797522   10.16  0.000   2.25   1.92   2.63


Log-Likelihood = -76.118
Test that all slopes are zero: G = 813.966, DF = 1, P-Value = 0.000

Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             14066.1   5  0.000
Deviance               73.7   5  0.000
Hosmer-Lemeshow     14066.1   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4      5      6      7  Total
live
  Obs      1      1     1   100    100    100    100    403
  Exp    0.0    0.2  11.3  88.7  100.8  101.0  101.0
dead
  Obs    100    100   100     1      1      1      1    304
  Exp  101.0  100.8  89.7  12.3    0.2    0.0    0.0
Total    101    101   101   101    101    101    101    707


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120800     98.6  Somers' D              0.98
Discordant     812      0.7  Goodman-Kruskal Gamma  0.99
Ties           900      0.7  Kendall's Tau-a        0.48
Total       122512    100.0
```

LT50 of 4-week cold acclimated *Arabidopsis* = -12.4419

Shandong: 4-Week cold acclimated in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     418  (Event)
          dead     287
          Total    705

Frequency: Frequency

Logistic Regression Table

                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -169.938    16.3893  -10.37  0.000
Temp       0.654246  0.0631355   10.36  0.000   1.92   1.70   2.18


Log-Likelihood = -99.023
Test that all slopes are zero: G = 754.807, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             1609.41   5  0.000
Deviance              43.14   5  0.000
Hosmer-Lemeshow     1609.41   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1     2     3     4      5      6      7  Total
live
  Obs      1     1    16   100    100    100    100    418
  Exp    0.0   1.2  24.0  90.3  100.5  101.0  101.0
dead
  Obs    100   100    83     1      1      1      1    287
  Exp  101.0  99.8  75.0  10.7    0.5    0.0    0.0
Total    101   101    99   101    101    101    101    705


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  117000     97.5  Somers' D              0.97
Discordant     838      0.7  Goodman-Kruskal Gamma  0.99
Ties          2128      1.8  Kendall's Tau-a        0.47
Total       119966    100.0
```

LT50 of 4-week cold acclimated Shandong = -13.2537

Yukon: 4-Week cold acclimated in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     513  (Event)
          dead     192
          Total    705

Frequency: Frequency

Logistic Regression Table

                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -162.122    15.4623  -10.48  0.000
Temp       0.635622  0.0606350   10.48  0.000   1.89   1.68   2.13


Log-Likelihood = -101.286
Test that all slopes are zero: G = 623.085, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             22232.5   5  0.000
Deviance               62.1   5  0.000
Hosmer-Lemeshow     22232.5   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value     1     2     3      4      5      6      7  Total
live
  Obs     1    12   100    100    100    100    100    513
  Exp   1.1  21.0  87.5  100.4  101.0  101.0  101.0
dead
  Obs   100    87     1      1      1      1      1    192
  Exp  99.9  78.0  13.5    0.6    0.0    0.0    0.0
Total   101    99   101    101    101    101    101    705


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant   95400     96.9  Somers' D              0.96
Discordant     852      0.9  Goodman-Kruskal Gamma  0.98
Ties          2244      2.3  Kendall's Tau-a        0.38
Total        98496    100.0
```

LT50 of 4-week cold acclimated Yukon = -17.9396

Arabidopsis: 3-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     430  (Event)
          dead     275
          Total    705

Frequency: Frequency

Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -155.093    14.8541  -10.44  0.000
Temp       0.598465  0.0573458   10.44  0.000   1.82   1.63   2.04


Log-Likelihood = -109.884
Test that all slopes are zero: G = 723.212, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             836.747   5  0.000
Deviance             34.516   5  0.000
Hosmer-Lemeshow     836.747   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1     2     3     4      5      6      7  Total
live
  Obs      1     1    28   100    100    100    100    430
  Exp    0.1   2.5  33.1  91.8  100.5  101.0  101.0
dead
  Obs    100   100    71     1      1      1      1    275
  Exp  100.9  98.5  65.9   9.2    0.5    0.0    0.0
Total    101   101    99   101    101    101    101    705


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  114600     96.9  Somers' D              0.96
Discordant     862      0.7  Goodman-Kruskal Gamma  0.99
Ties          2788      2.4  Kendall's Tau-a        0.46
Total       118250    100.0
```

LT50 of 3-week cold acclimated *Arabidopsis* = -13.8487

Shandong: 3-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit


Response Information

Variable  Value  Count
Response  live     493  (Event)
          dead     211
          Total    704

Frequency: Frequency


Logistic Regression Table
                                                 Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -109.480    9.25347  -11.83  0.000
Temp       0.427626  0.0361172   11.84  0.000   1.53   1.43   1.65


Log-Likelihood = -150.618
Test that all slopes are zero: G = 558.522, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             134.956   5  0.000
Deviance             18.080   5  0.003
Hosmer-Lemeshow     134.956   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                         Group
Value     1     2     3     4      5      6      7  Total
live
  Obs     1    22    70   100    100    100    100    493
  Exp   3.2  21.4  70.0  96.1  100.4  100.9  101.0
dead
  Obs   100    77    30     1      1      1      1    211
  Exp  97.8  77.6  30.0   4.9    0.6    0.1    0.0
Total   101    99   100   101    101    101    101    704


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant   97890     94.1  Somers' D              0.93
Discordant    1639      1.6  Goodman-Kruskal Gamma  0.97
Ties          4494      4.3  Kendall's Tau-a        0.39
Total       104023    100.0
```

LT50 0f 3-week cold acclimated Shandong =-16.9819

Yukon: 3-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit


Response Information

Variable  Value  Count
Response  live     571  (Event)
          dead     106
          Total    677

Frequency: Frequency

Logistic Regression Table
                                                 Odds     95% CI
Predictor      Coef    SE Coef      Z      P  Ratio  Lower  Upper
Constant   -147.971    15.6600  -9.45  0.000
Temp       0.589332  0.0623622   9.45  0.000   1.80   1.60   2.04


Log-Likelihood = -89.679
Test that all slopes are zero: G = 408.201, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             80995.9   5  0.000
Deviance              101.5   5  0.000
Hosmer-Lemeshow     80995.9   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value     1     2     3      4      5      6      7  Total
live
  Obs     1    70   100    100    100    100    100    571
  Exp  14.1  53.7  99.3  100.9  101.0  101.0  101.0
dead
  Obs   100     1     1      1      1      1      1    106
  Exp  86.9  17.3   1.7    0.1    0.0    0.0    0.0
Total   101    71   101    101    101    101    101    677


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant   57900     95.7  Somers' D              0.94
Discordant     756      1.2  Goodman-Kruskal Gamma  0.97
Ties          1870      3.1  Kendall's Tau-a        0.25
Total        60526    100.0
```

LT50 of 3-week cold acclimated Yukon = -21.9174

Arabidopsis: 2-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     452  (Event)
          dead     254
          Total    706

Frequency: Frequency


Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -145.129    13.8279  -10.50  0.000
Temp       0.562306  0.0535735   10.50  0.000   1.75   1.58   1.95


Log-Likelihood = -118.355
Test that all slopes are zero: G = 685.732, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             768.181   5  0.000
Deviance             30.758   5  0.000
Hosmer-Lemeshow     768.181   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1     2     3     4      5      6      7  Total
live
  Obs      1     1    50   100    100    100    100    452
  Exp    0.3   5.4  48.6  95.0  100.6  101.0  101.0
dead
  Obs    100   100    50     1      1      1      1    254
  Exp  100.7  95.6  51.4   6.0    0.4    0.0    0.0
Total    101   101   100   101    101    101    101    706


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  110600     96.3  Somers' D              0.96
Discordant     908      0.8  Goodman-Kruskal Gamma  0.98
Ties          3300      2.9  Kendall's Tau-a        0.44
Total       114808    100.0
```

LT50 of 2-week cold acclimated *Arabidopsis* =-14.9038

Shandong: 2-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     452  (Event)
          dead     254
          Total    706

Frequency: Frequency

Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -145.129    13.8279  -10.50  0.000
Temp       0.562306  0.0535735   10.50  0.000   1.75   1.58   1.95


Log-Likelihood = -118.355
Test that all slopes are zero: G = 685.732, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             768.181   5  0.000
Deviance             30.758   5  0.000
Hosmer-Lemeshow     768.181   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1     2     3     4      5      6      7  Total
live
  Obs      1     1    50   100    100    100    100    452
  Exp    0.3   5.4  48.6  95.0  100.6  101.0  101.0
dead
  Obs    100   100    50     1      1      1      1    254
  Exp  100.7  95.6  51.4   6.0    0.4    0.0    0.0
Total    101   101   100   101    101    101    101    706


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  110600     96.3  Somers' D              0.96
Discordant     908      0.8  Goodman-Kruskal Gamma  0.98
Ties          3300      2.9  Kendall's Tau-a        0.44
Total       114808    100.0
```

LT50 of 2-week cold acclimated Shandong =-14.9038

Yukon: 2-Week cold acclimated plants in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     500  (Event)
          dead     204
          Total    704

Frequency: Frequency


Logistic Regression Table

                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -97.9762    8.08597  -12.12  0.000
Temp       0.383292  0.0315992   12.13  0.000   1.47   1.38   1.56


Log-Likelihood = -165.738
Test that all slopes are zero: G = 516.066, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             67.1972   5  0.000
Deviance            22.5099   5  0.000
Hosmer-Lemeshow     67.1972   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                        Group
Value     1     2     3     4      5      6      7  Total
live
  Obs     1    33    66   100    100    100    100    500
  Exp   5.2  26.8  70.7  95.4  100.1  100.9  101.0
dead
  Obs   100    67    33     1      1      1      1    204
  Exp  95.8  73.2  28.3   5.6    0.9    0.1    0.0
Total   101   100    99   101    101    101    101    704


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant   94822     93.0  Somers' D              0.91
Discordant    2089      2.0  Goodman-Kruskal Gamma  0.96
Ties          5089      5.0  Kendall's Tau-a        0.37
Total       102000    100.0
```

LT50 of 2-week cold acclimated Yukon = -17.3824

Non acclimated *Arabidopsis* in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     369  (Event)
          dead     336
          Total    705

Frequency: Frequency


Logistic Regression Table

                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -156.212    15.1318  -10.32  0.000
Temp       0.595784  0.0576813   10.33  0.000   1.81   1.62   2.03


Log-Likelihood = -110.990
Test that all slopes are zero: G = 753.812, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             178.241   5  0.000
Deviance             28.629   5  0.000
Hosmer-Lemeshow     178.241   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                         Group
Value      1      2     3     4     5      6      7  Total
live
  Obs      1      1     1    66   100    100    100    369
  Exp    0.0    0.4   7.7  61.1  97.9  100.8  101.0
dead
  Obs    100    100   100    33     1      1      1    336
  Exp  101.0  100.6  93.3  37.9   3.1    0.2    0.0
Total    101    101   101    99   101    101    101    705


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120200     96.9  Somers' D              0.96
Discordant     806      0.7  Goodman-Kruskal Gamma  0.99
Ties          2978      2.4  Kendall's Tau-a        0.48
Total       123984    100.0
```

LT50 of non-acclimated *Arabidopsis* = -10.804

Non acclimated Shandong in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     369  (Event)
          dead     336
          Total    705

Frequency: Frequency

Logistic Regression Table

                                                 Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -156.212    15.1318  -10.32  0.000
Temp       0.595784  0.0576813   10.33  0.000   1.81   1.62   2.03


Log-Likelihood = -110.990
Test that all slopes are zero: G = 753.812, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             178.241   5  0.000
Deviance             28.629   5  0.000
Hosmer-Lemeshow     178.241   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4     5      6      7  Total
live
  Obs      1      1     1    66   100    100    100    369
  Exp    0.0    0.4   7.7  61.1  97.9  100.8  101.0
dead
  Obs    100    100   100    33     1      1      1    336
  Exp  101.0  100.6  93.3  37.9   3.1    0.2    0.0
Total    101    101   101    99   101    101    101    705


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120200     96.9  Somers' D              0.96
Discordant     806      0.7  Goodman-Kruskal Gamma  0.99
Ties          2978      2.4  Kendall's Tau-a        0.48
Total       123984    100.0
```

LT50 of non-acclimated Shandong = -10.804

Non-acclimated Yukon in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     332  (Event)
          dead     374
          Total    706

Frequency: Frequency


Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -160.319    15.5394  -10.32  0.000
Temp       0.607228  0.0588886   10.31  0.000   1.84   1.64   2.06


Log-Likelihood = -109.254
Test that all slopes are zero: G = 757.716, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             226.493   5  0.000
Deviance             30.756   5  0.000
Hosmer-Lemeshow     226.493   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4     5      6      7  Total
live
  Obs      1      1     1    29   100    100    100    332
  Exp    0.0    0.1   2.5  35.0  92.7  100.6  101.0
dead
  Obs    100    100   100    71     1      1      1    374
  Exp  101.0  100.9  98.5  65.0   8.3    0.4    0.0
Total    101    101   101   100   101    101    101    706


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120500     97.0  Somers' D              0.96
Discordant     809      0.7  Goodman-Kruskal Gamma  0.99
Ties          2859      2.3  Kendall's Tau-a        0.48
Total       124168    100.0
```

LT50 of non-acclimated yukon is -9.5075

Non-acclimated 4 d droughted *Arabidopsis* in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     304  (Event)
          dead     403
          Total    707

Frequency: Frequency


Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -215.026    21.1677  -10.16  0.000
Temp       0.810069  0.0797522   10.16  0.000   2.25   1.92   2.63


Log-Likelihood = -76.118
Test that all slopes are zero: G = 813.966, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             14066.1   5  0.000
Deviance               73.7   5  0.000
Hosmer-Lemeshow     14066.1   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2      3     4     5      6      7  Total
live
  Obs      1      1      1     1   100    100    100    304
  Exp    0.0    0.0    0.2  12.3  89.7  100.8  101.0
dead
  Obs    100    100    100   100     1      1      1    403
  Exp  101.0  101.0  100.8  88.7  11.3    0.2    0.0
Total    101    101    101   101   101    101    101    707


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120800     98.6  Somers' D              0.98
Discordant     812      0.7  Goodman-Kruskal Gamma  0.99
Ties           900      0.7  Kendall's Tau-a        0.48
Total       122512    100.0
```

LT 50 of non-acclimated, 4-da droughted *Arabidopsis* = -7.5584

Non-acclimated, 4-d droughted Shandong in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     386  (Event)
          dead     320
          Total    706

Frequency: Frequency


Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -172.016    16.7765  -10.25  0.000
Temp       0.658063  0.0641247   10.26  0.000   1.93   1.70   2.19


Log-Likelihood = -99.327
Test that all slopes are zero: G = 773.891, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             645.527   5  0.000
Deviance             40.155   5  0.000
Hosmer-Lemeshow     645.527   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4     5      6      7  Total
live
  Obs      1      1     1    83   100    100    100    386
  Exp    0.0    0.4   9.8  74.2  99.7  101.0  101.0
dead
  Obs    100    100   100    17     1      1      1    320
  Exp  101.0  100.6  91.2  25.8   1.3    0.0    0.0
Total    101    101   101   100   101    101    101    706


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120500     97.6  Somers' D              0.97
Discordant     809      0.7  Goodman-Kruskal Gamma  0.99
Ties          2211      1.8  Kendall's Tau-a        0.48
Total       123520    100.0
```

LT 50 of non-acclimated, 4-d droughted Shandong = -11.6021

Non-acclimated, 4-d droughted Yukon in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     336  (Event)
          dead     370
          Total    706

Frequency: Frequency


Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -157.412    15.2331  -10.33  0.000
Temp       0.596657  0.0577644   10.33  0.000   1.82   1.62   2.03


Log-Likelihood = -111.470
Test that all slopes are zero: G = 754.146, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             181.535   5  0.000
Deviance             28.782   5  0.000
Hosmer-Lemeshow     181.535   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4     5      6      7  Total
live
  Obs      1      1     1    33   100    100    100    336
  Exp    0.0    0.2   3.0  38.0  93.3  100.6  101.0
dead
  Obs    100    100   100    67     1      1      1    370
  Exp  101.0  100.8  98.0  62.0   7.7    0.4    0.0
Total    101    101   101   100   101    101    101    706


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120500     96.9  Somers' D              0.96
Discordant     809      0.7  Goodman-Kruskal Gamma  0.99
Ties          3011      2.4  Kendall's Tau-a        0.48
Total       124320    100.0
```

LT50 of non-acclimated, 4-d droughted Yukon = -9.1767

1-week cold-acclimated Aravidopsis in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     403  (Event)
          dead     304
          Total    707

Frequency: Frequency

Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -211.070    20.7842  -10.16  0.000
Temp       0.810069  0.0797522   10.16  0.000   2.25   1.92   2.63


Log-Likelihood = -76.118
Test that all slopes are zero: G = 813.966, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             14066.1   5  0.000
Deviance               73.7   5  0.000
Hosmer-Lemeshow     14066.1   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4      5      6      7  Total
live
  Obs      1      1     1   100    100    100    100    403
  Exp    0.0    0.2  11.3  88.7  100.8  101.0  101.0
dead
  Obs    100    100   100     1      1      1      1    304
  Exp  101.0  100.8  89.7  12.3    0.2    0.0    0.0
Total    101    101   101   101    101    101    101    707


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120800     98.6  Somers' D              0.98
Discordant     812      0.7  Goodman-Kruskal Gamma  0.99
Ties           900      0.7  Kendall's Tau-a        0.48
Total       122512    100.0
```

LT50 of 1-week cold-acclimated Arabodopsis = -12.4419

1-Week cold-acclimated Shandong in Shandong Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     403  (Event)
          dead     304
          Total    707

Frequency: Frequency

Logistic Regression Table

                                                  Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -211.070    20.7842  -10.16  0.000
Temp       0.810069  0.0797522   10.16  0.000   2.25   1.92   2.63


Log-Likelihood = -76.118
Test that all slopes are zero: G = 813.966, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             14066.1   5  0.000
Deviance               73.7   5  0.000
Hosmer-Lemeshow     14066.1   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4      5      6      7  Total
live
  Obs      1      1     1   100    100    100    100    403
  Exp    0.0    0.2  11.3  88.7  100.8  101.0  101.0
dead
  Obs    100    100   100     1      1      1      1    304
  Exp  101.0  100.8  89.7  12.3    0.2    0.0    0.0
Total    101    101   101   101    101    101    101    707


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120800     98.6  Somers' D              0.98
Discordant     812      0.7  Goodman-Kruskal Gamma  0.99
Ties           900      0.7  Kendall's Tau-a        0.48
Total       122512    100.0
```

LT50 of 1-week cold-acclimated Shandong = -12.4419

1-week cold-acclimated Yukon in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     403  (Event)
          dead     304
          Total    707

Frequency: Frequency


Logistic Regression Table
                                                    Odds     95% CI
Predictor      Coef    SE Coef       Z      P  Ratio  Lower  Upper
Constant   -211.070    20.7842  -10.16  0.000
Temp       0.810069  0.0797522   10.16  0.000   2.25   1.92   2.63


Log-Likelihood = -76.118
Test that all slopes are zero: G = 813.966, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             14066.1   5  0.000
Deviance               73.7   5  0.000
Hosmer-Lemeshow     14066.1   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value      1      2     3     4      5      6      7  Total
live
  Obs      1      1     1   100    100    100    100    403
  Exp    0.0    0.2  11.3  88.7  100.8  101.0  101.0
dead
  Obs    100    100   100     1      1      1      1    304
  Exp  101.0  100.8  89.7  12.3    0.2    0.0    0.0
Total    101    101   101   101    101    101    101    707


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant  120800     98.6  Somers' D              0.98
Discordant     812      0.7  Goodman-Kruskal Gamma  0.99
Ties           900      0.7  Kendall's Tau-a        0.48
Total       122512    100.0
```

LT50 of 1-week cold-acclimated Yukon = -12.4419

1-week cold-acclimated, 4-d droughted Shandong in Yukon Regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     640  (Event)
          dead      56
          Total    696

Frequency: Frequency

Logistic Regression Table

                                                 Odds     95% CI
Predictor      Coef    SE Coef      Z      P  Ratio  Lower  Upper
Constant   -81.5165    11.2475  -7.25  0.000
Temp       0.329712  0.0448802   7.35  0.000   1.39   1.27   1.52


Log-Likelihood = -118.110
Test that all slopes are zero: G = 153.388, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             323.212   5  0.000
Deviance             45.245   5  0.000
Hosmer-Lemeshow     323.212   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                          Group
Value     1     2     3      4      5      6      7  Total
live
  Obs    40   100   100    100    100    100    100    640
  Exp  50.6  87.9  98.2  100.4  100.9  101.0  101.0
dead
  Obs    50     1     1      1      1      1      1     56
  Exp  39.4  13.1   2.8    0.6    0.1    0.0    0.0
Total    90   101   101    101    101    101    101    696


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant   31200     87.1  Somers' D              0.83
Discordant    1440      4.0  Goodman-Kruskal Gamma  0.91
Ties          3200      8.9  Kendall's Tau-a        0.12
Total        35840    100.0
```

LT50 of non-acclimated, 4-d droughted Shandong = -25.3435

1-Week cold acclimated, 4-d droughted Yukon in Yukon regime

Binary Logistic Regression: Response versus Temp

```
Link Function: Logit

Response Information

Variable  Value  Count
Response  live     625  (Event)
          dead      80
          Total    705

Frequency: Frequency

Logistic Regression Table

                                                    Odds     95% CI
Predictor      Coef    SE Coef      Z      P  Ratio  Lower  Upper
Constant   -63.0576    7.41381  -8.51  0.000
Temp       0.254441  0.0294314   8.65  0.000   1.29   1.22   1.37


Log-Likelihood = -161.894
Test that all slopes are zero: G = 174.957, DF = 1, P-Value = 0.000


Goodness-of-Fit Tests

Method           Chi-Square  DF      P
Pearson             30.9940   5  0.000
Deviance            16.5897   5  0.005
Hosmer-Lemeshow     30.9940   5  0.000


Table of Observed and Expected Frequencies:
(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

                        Group
Value     1     2     3     4      5      6      7  Total
live
  Obs    50    75   100   100    100    100    100    625
  Exp  51.1  78.9  93.9  98.9  100.4  100.8  101.0
dead
  Obs    50    25     1     1      1      1      1     80
  Exp  48.9  21.1   7.1   2.1    0.6    0.2    0.0
Total   100   100   101   101    101    101    101    705


Measures of Association:
(Between the Response Variable and Predicted Probabilities)

Pairs       Number  Percent  Summary Measures
Concordant   42150     84.3  Somers' D              0.79
Discordant    2775      5.5  Goodman-Kruskal Gamma  0.88
Ties          5075     10.2  Kendall's Tau-a        0.16
Total        50000    100.0
```

LT50 fo 1-Wk cold acclimated, 4-d droughted Yukon = -25.1720

Appendix B: Statistical Output for Chapter 4

ANOVA results of growth parameters of 3 experimental Taxa in Yukon regime
Rosette leaves: Taxa (P≤0.0001); Taxa*DAS (P≤0.0001).
Rosette radius: Taxa (P≤0.0001); Taxa*DAS (P=0.024).
Leaf area: Taxa (P≤0.0001); Taxa*DAS (P≤0.0001)
Shoot dry weight: Taxa (P≤0.0001); Taxa*DAS (P≤0.0001).
SLA: Taxa (P=0.001); Taxa*DAS (P=0.002).
ULR: Taxa (P=0.22).
RGR: Taxa (P=0.0003); Taxa*DAS (P≤0.0001).

ANOVA results of growth parameters of 3 Taxa in Shandong regime
Rosette leaves: Taxa (P≤0.0001). Taxa*DAS (P≤0.0001).
Rosette radius: Taxa (P≤0.0001); Taxa*DAS (P=0.040).
Leaf area: Taxa (P≤0.0001); Taxa*DAS (P≤0.0001)
Shoot dry weight: Taxa (P≤0.0001); Taxa*DAS (P≤0.0001).
SLA: Taxa (P=0.002); Taxa*DAS (P<0.0001).
ULR: Taxa (P= 0.81);
RGR: Taxa (P=0.001); Taxa*DAS (P≤0.0001).

ANOVA of growth parameters of 3 Taxa in *Arabidopsis* regime
Rosette leaves: Taxa (P≤0.0001); Taxa*DAS (P≤0.0001).
Rosette radius: Taxa (P=<0.0001); Taxa*DAS (P=<0.0001)
Leaf area: Taxa (P=<0.0001); Taxa*DAS (P=<0.0001)
Shoot dry weight: Taxa (P=<0.0001); Taxa*DAS (P=<0.0001)
SLA: Taxa (P=<0.0001); Taxa*DAS (P=<0.0001)
ULR: Taxa (P=0.01);
RGR: Taxa (P=<0.0001); Taxa*DAS (P=<0.0001)

ANOVA results of growth parameters of *Arabidopsis* across 3 growth regimes
Rosette leaves: Regime (P≤0.0001); Regime*DAS (P≤0.0001).
Rosette radius: Regime (P≤0.0001); Regime*DAS (P≤0.0003).
Leaf area: Regime (P≤0.0001); Regime*DAS (P≤0.0001)
Shoot dry weight: Regime (P≤0.0001); Regime*DAS (P≤0.0001).
SLA: Regime (P≤0.0001); Regime*DAS (P=0.001).
ULR: Regime (P=0.004); Regime*DAS (P≤0.0001).
RGR: Regime (P=0.08); Regime*DAS (P=0.09).

ANOVA results of growth parameters of *Thellungiella* Yukon Taxa across 3 growth regimes
Rosette leaves: Regime (P≤0.0001); Regime*DAS (P≤0.0001).
Rosette radius: Regime (P≤0.0001); Regime*DAS (P<0.0001).
Leaf area: Regime (P≤0.0001); Regime*DAS (P≤0.0001).
Shoot dry weight: Regime (P≤.0001); Regime*DAS (P≤.00001).
SLA: Regime (P≤0.0001); Regime*DAS (P=0.022).
ULR: Regime (P=0.03);
RGR: Regime (P=0.001); Regime*DAS (P=0.001).

ANOVA results of growth parameters of Shandong Taxa across 3 growth regimes
Rosette leaves: Regime (P≤0.0001); Regime*DAS (P≤0.0001).
Rosette radius: Regime (P≤0.0001); Regime*DAS (P≤0.008).
Leaf area: Regime (P≤0.0001); Regime*DAS (P≤0.0001).
Shoot dry weight: Regime (P<0.0001); Regime*DAS (P<0.0001).
SLA: Regime (P≤0.0001); Regime*DAS (P<0.0001).
ULR: Regime (P=0.15).
RGR: Regime (P=0.33); Regime*DAS (P<0.0001).

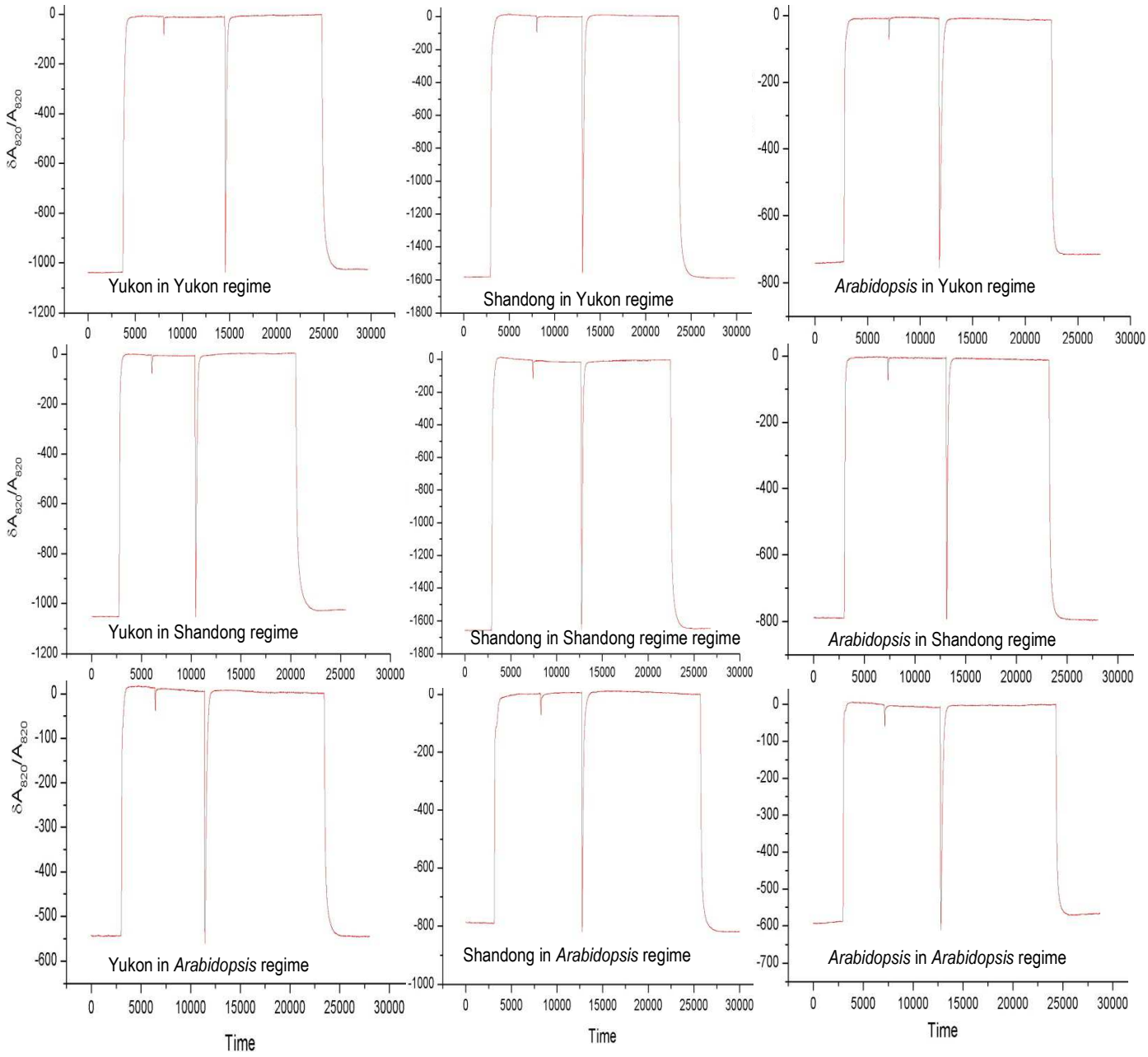
Appendix C: Growth conditions used in studies of *Thellungiella* and *Arabidopsis*

Plant Taxa	Temperature (°C) day/night	Light/dark period (h)	PPFD (µmol photons $m^{-2}s^{-1}$)	Reference
A. thaliana	25/20	8/16	150	Armstrong et al. (2006)
24 *Arabidopsis* accessions	20/6	16/8	145	Cross et al. (2006)
A. thaliana	20/18	14/10	180	Golan et al. (2006)
A. thaliana	23/18	8/16	150	Goulas et al. (2006)
A. thaliana	23/23	8/16	110	Gray et al. (2003)
3 *Arabidopsis* accessions	22/22	16/8	80	Passardi et al. (2006)
A. thaliana	25/25	8/16	150	Rosso et al. (2006)
A. thaliana	22/22	16/8	150-200	Sweetlove et al. (2006)
A. thaliana and *T. hlophila*	23/23	16/8	NA	Fang et al. (2006)
A. thaliana and *T. hlophila*	21/8 (greenhouse) 22/19 (chamber)	16/8	250	Inan et al. (2004)
A. thaliana and *T. hlophila*	NA	12/12	150 (chamber) 300 (glass house)	M'rah et al. (2006)
A. thaliana and *T. hlophila*	22/22	NA	200	Teusink et al. (2002)
T. salsuginea (Yukon)	22/10	21/3	250	Wong et al. (2005, 2006) Griffith et al. (2007)

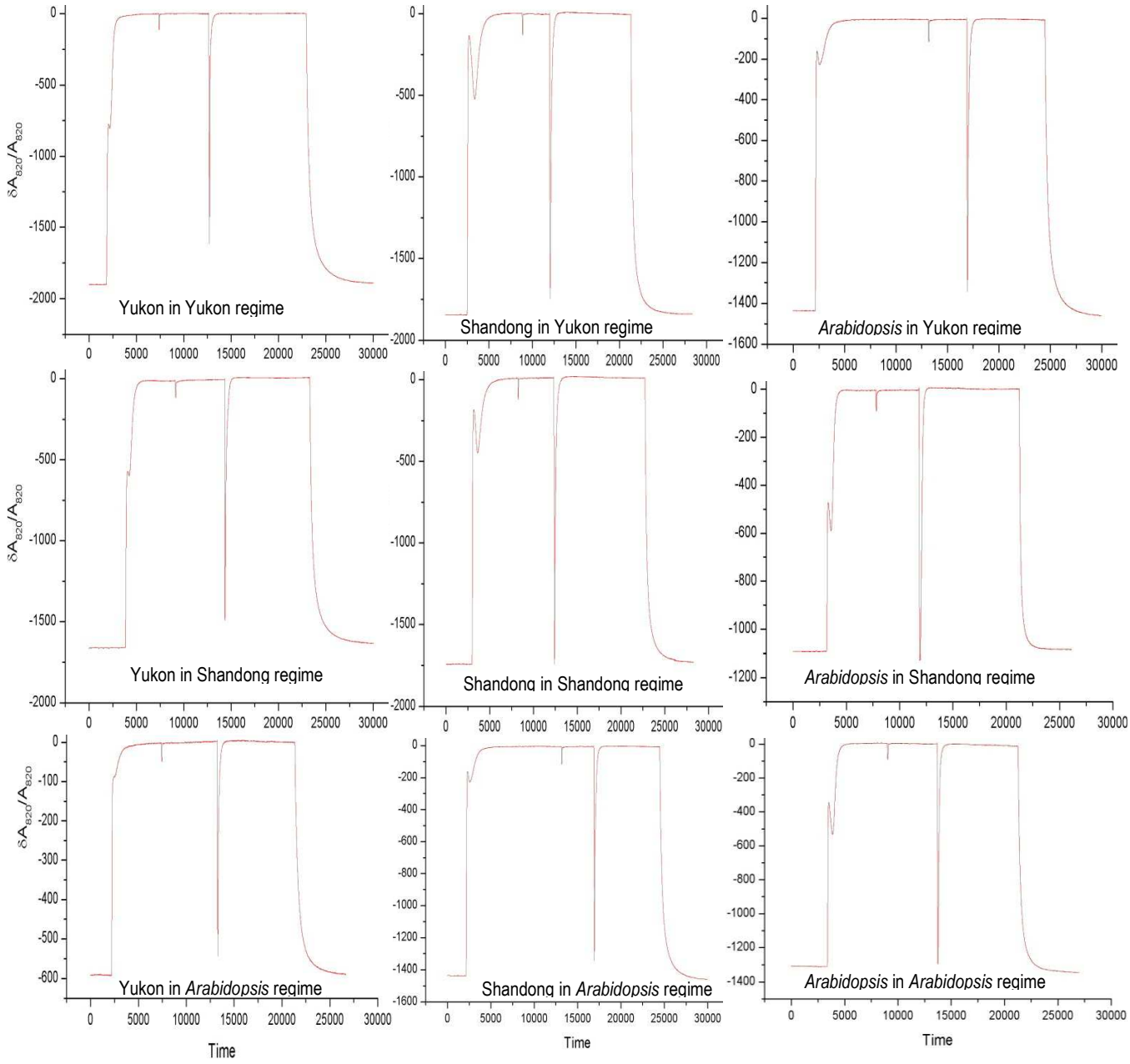
NA, data not provided

Appendix D: Raw Data Traces for P_{700} Measurements:

1. P_{700}^{+} traces of non-acclimated plants measured at 20°C



2. P_{700}^{+} traces of cold acclimated plants measured at 4.5°C



Appendix E: Statistical Output for Chapter 5

ANOVA of photoinhibition and recovery of Yukon, Shandong and *Arabidopsis*. General Linear Model: Control, Inhibition, ... versus Regime, Acclimation, ...

Factor	Type	Levels	Values
Regime	fixed	3	At-regim, Sh-Regime, Yu-Regime
Acclimation	fixed	2	Cold-acclimated, Non-acclimated
Ecotype	fixed	3	*Arabidopsis*, Shandong, Yukon
Reps	random	4	1, 2, 3, 4

Analysis of Variance for Control, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regime	2	0.0046739	0.0046739	0.0023370	46.06	0.000
Acclimation	1	0.0102096	0.0102096	0.0102096	201.24	0.000
Ecotype	2	0.0060965	0.0060965	0.0030482	60.08	0.000
Reps	3	0.0000595	0.0000595	0.0000198	0.39	0.760
Regime*Acclimation	2	0.0047548	0.0047548	0.0023774	46.86	0.000
Regime*Ecotype	4	0.0034532	0.0034532	0.0008633	17.02	0.000
Acclimation*Ecotype	2	0.0082028	0.0082028	0.0041014	80.84	0.000
Regime*Acclimation*Ecotype	4	0.0062505	0.0062505	0.0015626	30.80	0.000
Error	51	0.0025874	0.0025874	0.0000507		
Total	71	0.0462882				

S = 0.00712276 R-Sq = 94.41% R-Sq(adj) = 92.22%

Unusual Observations for Control

Obs	Control	Fit	SE Fit	Residual	St Resid
7	0.763614	0.776958	0.003847	-0.013344	-2.23 R
17	0.753107	0.767614	0.003847	-0.014507	-2.42 R
41	0.818121	0.792940	0.003847	0.025181	4.20 R
43	0.775210	0.790729	0.003847	-0.015519	-2.59 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Inhibition, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regime	2	0.084413	0.084413	0.042206	128.76	0.000
Acclimation	1	0.343724	0.343724	0.343724	1048.61	0.000
Ecotype	2	0.068707	0.068707	0.034353	104.80	0.000
Reps	3	0.000233	0.000233	0.000078	0.24	0.870
Regime*Acclimation	2	0.000024	0.000024	0.000012	0.04	0.964
Regime*Ecotype	4	0.054478	0.054478	0.013620	41.55	0.000
Acclimation*Ecotype	2	0.015500	0.015500	0.007750	23.64	0.000
Regime*Acclimation*Ecotype	4	0.003152	0.003152	0.000788	2.40	0.062
Error	51	0.016717	0.016717	0.000328		
Total	71	0.586948				

S = 0.0181050 R-Sq = 97.15% R-Sq(adj) = 96.03%

Unusual Observations for Inhibition

```
Obs  Inhibition       Fit    SE Fit   Residual  St Resid
 21    0.644434  0.675410  0.009778  -0.030976     -2.03 R
 25    0.598530  0.562258  0.009778   0.036272      2.38 R
 26    0.525308  0.560305  0.009778  -0.034997     -2.30 R
 49    0.559154  0.521589  0.009778   0.037565      2.47 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Recovery, using Adjusted SS for Tests

Source                       DF     Seq SS     Adj SS     Adj MS      F      P
Regime                        2  0.0067446  0.0067446  0.0033723  96.63  0.000
Acclimation                   1  0.0002102  0.0002102  0.0002102   6.02  0.018
Ecotype                       2  0.0064762  0.0064762  0.0032381  92.78  0.000
Reps                          3  0.0000890  0.0000890  0.0000297   0.85  0.473
Regime*Acclimation            2  0.0015974  0.0015974  0.0007987  22.89  0.000
Regime*Ecotype                4  0.0010123  0.0010123  0.0002531   7.25  0.000
Acclimation*Ecotype           2  0.0041700  0.0041700  0.0020850  59.74  0.000
Regime*Acclimation*Ecotype    4  0.0005342  0.0005342  0.0001336   3.83  0.009
Error                        51  0.0017799  0.0017799  0.0000349
Total                        71  0.0226139


S = 0.00590768   R-Sq = 92.13%   R-Sq(adj) = 89.04%


Unusual Observations for Recovery

Obs  Recovery       Fit    SE Fit   Residual  St Resid
  5  0.797005  0.779669  0.003191   0.017336      3.49 R
  7  0.764728  0.778325  0.003191  -0.013597     -2.73 R
 15  0.771656  0.759943  0.003191   0.011713      2.36 R
 18  0.797427  0.780929  0.003191   0.016498      3.32 R
 69  0.818470  0.829882  0.003191  -0.011413     -2.30 R

R denotes an observation with a large standardized residual.
```

Two-way ANOVA: Control versus Acclimation, Ecotype

```
Source       DF         SS         MS      F      P
Acclimation   1  0.0102096  0.0102096  30.94  0.000
Ecotype       2  0.0060965  0.0030482   9.24  0.000
Interaction   2  0.0082028  0.0041014  12.43  0.000
Error        66  0.0217793  0.0003300
Total        71  0.0462882

S = 0.01817   R-Sq = 52.95%   R-Sq(adj) = 49.38%


                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Acclimation       Mean --------+---------+---------+---------+-
Cold-acclima  0.778082 (-----*-----)
Non-acclimat  0.801898                          (-----*-----)
                       --------+---------+---------+---------+-
                             0.780     0.790     0.800     0.810
```

```
                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Ecotype          Mean  ----+---------+---------+---------+-----
Arabidopsis   0.783177  (------*-------)
Shandong      0.802999                      (------*------)
Yukon         0.783794  (-------*------)
                     ----+---------+---------+---------+-----
                       0.780     0.790     0.800     0.810
```

Two-way ANOVA: Inhibition versus Acclimation, Ecotype

```
Source       DF        SS        MS       F      P
Acclimation   1  0.343724  0.343724  142.66  0.000
Ecotype       2  0.068707  0.034353   14.26  0.000
Interaction   2  0.015500  0.007750    3.22  0.046
Error        66  0.159017  0.002409
Total        71  0.586948

S = 0.04909   R-Sq = 72.91%   R-Sq(adj) = 70.86%
```

```
                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Acclimation       Mean  --+---------+---------+---------+-------
Cold-acclima  0.645073                              (--*--)
Non-acclimat  0.506885  (--*---)
                      --+---------+---------+---------+-------
                      0.500     0.550     0.600     0.650
```

```
                     Individual 95% CIs For Mean Based on Pooled StDev
Ecotype          Mean  ---------+---------+---------+---------+
Arabidopsis   0.532981  (------*-----)
Shandong      0.604169                       (-----*------)
Yukon         0.590787                  (------*------)
                     ---------+---------+---------+---------+
                            0.540     0.570     0.600     0.630
```

Two-way ANOVA: Recovery versus Acclimation, Ecotype

```
Source       DF         SS         MS      F      P
Acclimation   1  0.0002102  0.0002102   1.18  0.281
Ecotype       2  0.0064762  0.0032381  18.18  0.000
Interaction   2  0.0041700  0.0020850  11.70  0.000
Error        66  0.0117575  0.0001781
Total        71  0.0226139

S = 0.01335   R-Sq = 48.01%   R-Sq(adj) = 44.07%
```

```
                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Acclimation       Mean  ----+---------+---------+---------+-----
Cold-acclima  0.804448            (-----------*-----------)
Non-acclimat  0.801030  (-----------*-----------)
                      ----+---------+---------+---------+-----
                        0.7980    0.8015    0.8050    0.8085
```

```
                     Individual 95% CIs For Mean Based on
```

```
                      Pooled StDev
Ecotype          Mean   -+---------+---------+---------+--------
Arabidopsis  0.798215       (----*-----)
Shandong     0.815936                       (-----*----)
Yukon        0.794067   (----*-----)
                        -+---------+---------+---------+--------
                       0.790     0.800     0.810     0.820
```

Two-way ANOVA: Control versus Regime, Ecotype

```
Source       DF         SS         MS     F      P
Regime        2  0.0046739  0.0023370  4.59  0.014
Ecotype       2  0.0060965  0.0030482  5.99  0.004
Interaction   4  0.0034532  0.0008633  1.70  0.162
Error        63  0.0320645  0.0005090
Total        71  0.0462882

S = 0.02256   R-Sq = 30.73%   R-Sq(adj) = 21.93%


                     Individual 95% CIs For Mean Based on Pooled StDev
Regime         Mean  ---------+---------+---------+---------+
At-regim   0.779765  (--------*--------)
Sh-Regime  0.799456                    (--------*---------)
Yu-Regime  0.790749            (--------*--------)
                     ---------+---------+---------+---------+
                            0.780     0.790     0.800     0.810


                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Ecotype          Mean  ------+---------+---------+---------+---
Arabidopsis  0.783177  (--------*--------)
Shandong     0.802999                      (--------*--------)
Yukon        0.783794   (--------*--------)
                       ------+---------+---------+---------+---
                          0.780     0.790     0.800     0.810
```

Two-way ANOVA: Inhibition versus Regime, Ecotype

```
Source       DF         SS         MS     F      P
Regime        2  0.084413  0.0422063  7.01  0.002
Ecotype       2  0.068707  0.0343535  5.71  0.005
Interaction   4  0.054478  0.0136196  2.26  0.072
Error        63  0.379350  0.0060214
Total        71  0.586948

S = 0.07760   R-Sq = 35.37%   R-Sq(adj) = 27.16%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Regime         Mean  -----+---------+---------+---------+----
At-regim   0.529673  (------*-------)
Sh-Regime  0.586867               (-------*-------)
Yu-Regime  0.611397                     (-------*-------)
```

```
                    -----+---------+---------+---------+----
                       0.520     0.560     0.600     0.640


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Ecotype          Mean  -------+---------+---------+---------+--
Arabidopsis  0.532981  (--------*--------)
Shandong     0.604169                     (--------*--------)
Yukon        0.590787                  (--------*--------)
                      -------+---------+---------+---------+--
                           0.525     0.560     0.595     0.630
```

Two-way ANOVA: Recovery versus Regime, Ecotype

```
Source       DF         SS         MS      F      P
Regime        2  0.0067446  0.0033723  25.35  0.000
Ecotype       2  0.0064762  0.0032381  24.34  0.000
Interaction   4  0.0010123  0.0002531   1.90  0.121
Error        63  0.0083808  0.0001330
Total        71  0.0226139

S = 0.01153   R-Sq = 62.94%   R-Sq(adj) = 58.23%


                    Individual 95% CIs For Mean Based on Pooled StDev
Regime         Mean  ---------+---------+---------+---------+
At-regim   0.789188  (----*-----)
Sh-Regime  0.807845                       (-----*-----)
Yu-Regime  0.811185                           (-----*-----)
                    ---------+---------+---------+---------+
                           0.7920    0.8000    0.8080    0.8160


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Ecotype          Mean  ---+---------+---------+---------+------
Arabidopsis  0.798215       (-----*-----)
Shandong     0.815936                           (-----*-----)
Yukon        0.794067  (-----*----)
                      ---+---------+---------+---------+------
                       0.7920    0.8000    0.8080    0.8160
```

ANOVA of P700 measurement of Yukon, Shandong and *Arabidopsis* grown under various growth regimes

General Linear Model: Absorbance versus Acclimation, Regime, ...

Factor	Type	Levels	Values
Acclimation	fixed	2	Cold-acclim, Non-acclimated
Regime	fixed	3	At-Regim, Sh-Regim, Yu-Regim
MeasurTemp	fixed	2	5-degree, RoomTemp
Ecotype	fixed	3	*Arabidopsis*, Shandong, Yukon
Rep	random	10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10

Analysis of Variance for Absorbance, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Acclimation	1	3650806	3236301	3236301	104.93	0.000
Regime	2	7454417	7965021	3982511	129.12	0.000
MeasurTemp	1	643806	446455	446455	14.48	0.000
Ecotype	2	8463152	8298225	4149113	134.52	0.000
Rep	9	511223	186940	20771	0.67	0.732
Acclimation*Regime*MeasurTemp	2	1467	525	263	0.01	0.992
Acclimation*MeasurTemp*Ecotype	2	23383	29130	14565	0.47	0.625
Acclimation*Regime	2	185937	185937	92968	3.01	0.052
Acclimation*MeasurTemp	1	577	192	192	0.01	0.937
Acclimation*Ecotype	2	558878	584395	292198	9.47	0.000
Regime*MeasurTemp	2	30640	30640	15320	0.50	0.610
Regime*Ecotype	4	4989366	4989366	1247342	40.44	0.000
MeasurTemp*Ecotype	2	11632	11632	5816	0.19	0.828
Error	150	4626413	4626413	30843		
Total	182	31151696				

S = 175.621 R-Sq = 85.15% R-Sq(adj) = 81.98%

Unusual Observations for Absorbance

Obs	Absorbance	Fit	SE Fit	Residual	St Resid	
19	621.00	296.80	74.77	324.20	2.04	R
80	894.00	894.00	175.62	0.00	*	X
81	984.00	984.00	175.62	0.00	*	X
82	789.00	789.00	175.62	0.00	*	X
92	1304.00	1677.41	63.91	-373.41	-2.28	R
113	1404.00	1776.38	63.91	-372.38	-2.28	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

General Linear Model: e-donorPool versus Acclimation, Regime, ...

Factor	Type	Levels	Values
Acclimation	fixed	2	Cold-acclim, Non-acclimated
Regime	fixed	3	At-Regim, Sh-Regim, Yu-Regim
MeasurTemp	fixed	2	5-degree, RoomTemp
Ecotype	fixed	3	*Arabidopsis*, Shandong, Yukon
Rep	random	10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10

Analysis of Variance for e-donorPool, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Acclimation	1	4093.8	3849.2	3849.2	15.12	0.000
Regime	2	1483.4	748.4	374.2	1.47	0.233
MeasurTemp	1	879.6	1078.0	1078.0	4.23	0.041
Ecotype	2	1316.1	466.7	233.3	0.92	0.402
Rep	9	1278.5	1248.6	138.7	0.54	0.840
Acclimation*Regime*MeasurTemp	2	621.9	590.5	295.3	1.16	0.316
Acclimation*MeasurTemp*Ecotype	2	417.6	213.7	106.8	0.42	0.658
Acclimation*Regime	2	885.7	885.7	442.9	1.74	0.179
Acclimation*MeasurTemp	1	23.0	17.9	17.9	0.07	0.791
Acclimation*Ecotype	2	3067.8	2614.0	1307.0	5.13	0.007
Regime*MeasurTemp	2	709.8	709.8	354.9	1.39	0.251
Regime*Ecotype	4	5202.3	5202.3	1300.6	5.11	0.001
MeasurTemp*Ecotype	2	2073.7	2073.7	1036.8	4.07	0.019
Error	150	38191.3	38191.3	254.6		
Total	182	60244.6				

S = 15.9565 R-Sq = 36.61% R-Sq(adj) = 23.08%

Unusual Observations for e-donorPool

Obs	e-donorPool	Fit	SE Fit	Residual	St Resid	
13	63.087	28.912	6.794	34.175	2.37	R
80	35.045	35.045	15.956	-0.000	*	X
81	60.463	60.463	15.956	-0.000	*	X
82	59.832	59.832	15.956	-0.000	*	X
95	74.274	45.099	6.658	29.175	2.01	R
97	27.289	67.208	5.806	-39.919	-2.69	R
98	26.236	65.957	5.806	-39.721	-2.67	R
99	131.028	70.500	5.806	60.528	4.07	R
101	170.515	67.499	6.658	103.016	7.10	R
148	86.178	35.217	5.806	50.961	3.43	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

ANOVA Results (p value) of different pigment variables of *Thellungiella* Yukon, *Thellungiella* Shandong and *Arabidopsis thaliana* grown under various growth regimes.

Source	ChlW	CarW	ChlLA	CarLA	Chl a/b	Chl/Car
Acclimation	<.0001	<.0001	0.0363	<.0001	0.0035	<.0001
Ecotype	<.0001	<.0001	<.0001	<.0001	0.9102	0.3893
Regime	0.0371	0.1883	<.0001	<.0001	<.0001	0.0603
Rep	0.2704	0.2789	0.5574	0.6776	0.5435	0.1466
Acclimation*Ecotype	<.0001	<.0001	0.0007	0.0012	<.0001	0.0183
Acclimation*Regime	0.0398	0.0075	0.0530	0.0043	0.0990	0.4673
Acclimation*Rep	0.2197	0.0044	0.8266	0.5334	0.2157	0.1760
Regime*Ecotype	0.0142	0.0004	0.0004	0.0003	0.0001	0.0194
Ecotype*Rep	0.7034	0.1233	0.8225	0.5090	0.1548	0.7522
Regime*Rep	0.6608	0.2532	0.9821	0.7591	0.5819	0.9833
Acclim*Regime*Ecotyp	0.0054	0.0394	0.0003	0.0146	0.0021	0.0011
Acclimat*Ecotype*Rep	0.0607	0.3352	0.0961	0.2926	0.2165	0.3209
Acclimati*Regime*Rep	0.4399	0.0153	0.8329	0.6857	0.5611	0.6874
Regime*Ecotype*Rep	0.1655	0.0656	0.414	0.6915	0.1760	0.4947

Appendix F: Statistical Output for Chapter 6

ANOVA of Respiration measurement

Gas phase measurement - Yukon Regime

General Linear Model: Respiration versus Acclimation, Ecotype, ...

(Temperature refers to the measurement temperature)

Factor	Type	Levels	Values
Acclimation	fixed	2	Cold-acclim, Non-acclim
Ecotype	fixed	3	*Arabidopsis*, Shandong, Yukon
Temperature	fixed	2	22C, 4.5C
Rep	random	6	1, 2, 3, 4, 5, 6

Analysis of Variance for Respiration, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Acclimation	1	75.253	75.253	75.253	34.11	0.000
Ecotype	2	70.567	70.567	35.283	15.99	0.000
Temperature	1	485.245	485.245	485.245	219.95	0.000
Rep	5	11.665	11.665	2.333	1.06	0.394
Acclimation*Ecotype	2	8.590	8.590	4.295	1.95	0.152
Acclimation*Temperature	1	42.350	42.350	42.350	19.20	0.000
Ecotype*Temperature	2	25.296	25.296	12.648	5.73	0.005
Acclimation*Ecotype*Temperature	2	11.153	11.153	5.577	2.53	0.089
Error	55	121.337	121.337	2.206		
Total	71	851.456				

S = 1.48531 R-Sq = 85.75% R-Sq(adj) = 81.60%

Unusual Observations for Respiration

Obs	Respiration	Fit	SE Fit	Residual	St Resid	
25	12.8957	9.8008	0.7217	3.0948	2.38	R
39	15.3247	12.6032	0.7217	2.7215	2.10	R
61	10.9356	14.2244	0.7217	-3.2888	-2.53	R

R denotes an observation with a large standardized residual.

Yukon Regime, non-acclimated, warm&cold temperature measurement

Two-way ANOVA: Respiration versus Ecotype, Temperature

```
Source       DF       SS       MS      F      P
Ecotype       2   33.255   16.627   7.59  0.002
Temperature   1  120.445  120.445  54.97  0.000
Interaction   2    2.632    1.316   0.60  0.555
Error        30   65.733    2.191
Total        35  222.064

S = 1.480   R-Sq = 70.40%   R-Sq(adj) = 65.47%


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Ecotype         Mean ---+---------+---------+---------+------
Arabidopsis  5.85491     (--------*-------)
Shandong     5.59151  (--------*--------)
Yukon        7.74924                       (-------*--------)
                     ---+---------+---------+---------+------
                      5.0       6.0       7.0       8.0


                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Temperature     Mean ----+---------+---------+---------+-----
22C          8.22767                          (----*----)
4.5C         4.56943  (---*----)
                     ----+---------+---------+---------+-----
                       4.5       6.0       7.5       9.0
```

Yukon Regime, Non-acclimated warm-temperature measured respiration results

One-way ANOVA: Respiration versus Ecotype

```
Source   DF     SS     MS     F      P
Ecotype   2  21.42  10.71  4.88  0.023
Error    15  32.93   2.20
Total    17  54.35

S = 1.482   R-Sq = 39.41%   R-Sq(adj) = 31.33%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level        N   Mean  StDev  --+---------+---------+---------+-------
Arabidopsis  6  7.953  1.499        (--------*--------)
Shandong     6  7.051  1.080  (--------*--------)
Yukon        6  9.680  1.781                     (--------*-------)
                              --+---------+---------+---------+-------
                              6.0       7.5       9.0      10.5

Pooled StDev = 1.482


Tukey 95% Simultaneous Confidence Intervals
```

```
All Pairwise Comparisons among Levels of Ecotype

Individual confidence level = 97.97%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center  Upper  ---------+---------+---------+---------+
Shandong  -3.122  -0.902  1.318         (-------*--------)
Yukon     -0.493   1.727  3.947                   (--------*--------)
                                  ---------+---------+---------+---------+
                                        -2.5       0.0       2.5       5.0


Ecotype = Shandong subtracted from:

Ecotype  Lower  Center  Upper  ---------+---------+---------+---------+
Yukon    0.409   2.629  4.849                      (--------*-------)
                               ---------+---------+---------+---------+
                                     -2.5       0.0       2.5       5.0


Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Simultaneous confidence level = 88.31%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center  Upper  --------+---------+---------+---------+-
Shandong  -2.725  -0.902  0.921         (------*-------)
Yukon     -0.096   1.727  3.551                    (------*------)
                                  --------+---------+---------+---------+-
                                       -2.5       0.0       2.5       5.0


Ecotype = Shandong subtracted from:

Ecotype  Lower  Center  Upper  --------+---------+---------+---------+-
Yukon    0.806   2.629  4.453                      (-------*------)
                               --------+---------+---------+---------+-
                                    -2.5       0.0       2.5       5.0
```

Yukon Regime, Non-acclimated Cold-temperature measured respiration results

One-way ANOVA: Respiration versus Ecotype

```
Source   DF     SS    MS     F      P
Ecotype   2  14.47  7.23  3.31  0.065
Error    15  32.80  2.19
Total    17  47.27

S = 1.479   R-Sq = 30.61%   R-Sq(adj) = 21.36%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level        N   Mean  StDev  ---------+---------+---------+---------+
```

```
Arabidopsis  6  3.757  1.751  (---------*----------)
Shandong     6  4.132  0.704     (---------*----------)
Yukon        6  5.819  1.732                   (---------*----------)
                             ---------+---------+---------+---------+
                                    3.6       4.8       6.0       7.2

Pooled StDev = 1.479


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Individual confidence level = 97.97%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center  Upper  ------+---------+---------+---------+---
Shandong  -1.840   0.375  2.591          (--------*-------)
Yukon     -0.154   2.062  4.277                  (--------*--------)
                             ------+---------+---------+---------+---
                                -2.5       0.0       2.5       5.0


Ecotype = Shandong subtracted from:

Ecotype   Lower  Center  Upper  ------+---------+---------+---------+---
Yukon    -0.529   1.686  3.902               (--------*--------)
                             ------+---------+---------+---------+---
                                -2.5       0.0       2.5       5.0


Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Simultaneous confidence level = 88.31%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center  Upper  --------+---------+---------+---------+-
Shandong  -1.445   0.375  2.195            (--------*--------)
Yukon      0.242   2.062  3.881                     (--------*--------)
                             --------+---------+---------+---------+-
                                  -2.0       0.0       2.0       4.0


Ecotype = Shandong subtracted from:

Ecotype   Lower  Center  Upper  --------+---------+---------+---------+-
Yukon    -0.133   1.686  3.506                   (--------*---------)
                             --------+---------+---------+---------+-
                                  -2.0       0.0       2.0       4.0
```

Yukon Regime Cold acclimated, warm & cold-temperature measured results

(Temperature refers to the measurement temperature)

Two-way ANOVA: Respiration versus Ecotype, Temperature

```
Source       DF       SS       MS       F      P
Ecotype       2   45.902   22.951   10.24  0.000
Temperature   1  407.151  407.151  181.58  0.000
Interaction   2   33.817   16.909    7.54  0.002
Error        30   67.269    2.242
Total        35  554.139

S = 1.497   R-Sq = 87.86%   R-Sq(adj) = 85.84%


                      Individual 95% CIs For Mean Based on Pooled StDev
Ecotype         Mean    +---------+---------+---------+---------
Arabidopsis  8.82799                    (-------*------)
Shandong     6.90863    (-------*------)
Yukon        9.59306                              (------*------)
                      +---------+---------+---------+---------
                     6.0       7.2       8.4       9.6


                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Temperature     Mean  ---+---------+---------+---------+------
22C          11.8062                              (--*--)
4.5C          5.0802  (--*--)
                      ---+---------+---------+---------+------
                       5.0       7.5      10.0      12.5
```

Yukon Regime Cold acclimated, warm-temperature measured results

One-way ANOVA: Respiration versus Ecotype

```
Source   DF      SS     MS      F      P
Ecotype   2   78.56  39.28  11.54  0.001
Error    15   51.05   3.40
Total    17  129.62

S = 1.845   R-Sq = 60.61%   R-Sq(adj) = 55.36%


Level        N    Mean  StDev
Arabidopsis  6  12.267  2.173
Shandong     6   9.049  1.046
Yukon        6  14.103  2.097

             Individual 95% CIs For Mean Based on Pooled StDev
Level          +---------+---------+---------+---------
Arabidopsis                (-----*-----)
Shandong       (-----*------)
Yukon                            (-----*------)
               +---------+---------+---------+---------
             7.5      10.0      12.5      15.0
```

```
Pooled StDev = 1.845


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Individual confidence level = 97.97%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center   Upper     +---------+---------+---------+---------
Shandong  -5.982  -3.218  -0.454          (------*------)
Yukon     -0.927   1.837   4.601                    (------*------)
                                     +---------+---------+---------+---------
                                   -8.0      -4.0       0.0       4.0


Ecotype = Shandong subtracted from:

Ecotype  Lower  Center  Upper     +---------+---------+---------+---------
Yukon    2.291   5.055  7.819                        (------*------)
                                  +---------+---------+---------+---------
                                -8.0      -4.0       0.0       4.0


Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Simultaneous confidence level = 88.31%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center   Upper  --------+---------+---------+---------+-
Shandong  -5.489  -3.218  -0.948      (-----*-----)
Yukon     -0.434   1.837   4.107                (-----*----)
                                  --------+---------+---------+---------+-
                                       -4.0       0.0       4.0       8.0


Ecotype = Shandong subtracted from:

Ecotype  Lower  Center  Upper  --------+---------+---------+---------+-
Yukon    2.785   5.055  7.325                      (-----*----)
                                --------+---------+---------+---------+-
                                     -4.0       0.0       4.0       8.0
```

Yukon Regime Cold acclimated, Cold-temperature measured results

One-way ANOVA: Respiration versus Ecotype

```
Source   DF     SS    MS     F      P
Ecotype   2   1.16  0.58  0.53  0.597
Error    15  16.21  1.08
Total    17  17.37

S = 1.040   R-Sq = 6.65%   R-Sq(adj) = 0.00%
```

```
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level        N   Mean  StDev  -----+---------+---------+---------+----
Arabidopsis  6  5.389  1.167          (------------*-----------)
Shandong     6  4.769  0.966  (------------*-----------)
Yukon        6  5.083  0.973       (------------*-----------)
                           -----+---------+---------+---------+----
                              4.20      4.90      5.60      6.30

Pooled StDev = 1.040


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Individual confidence level = 97.97%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center  Upper  --------+---------+---------+---------+-
Shandong  -2.178  -0.621  0.937  (------------*-----------)
Yukon     -1.864  -0.307  1.251    (------------*-----------)
                                --------+---------+---------+---------+-
                                     -1.2       0.0       1.2       2.4


Ecotype = Shandong subtracted from:

Ecotype   Lower  Center  Upper  --------+---------+---------+---------+-
Yukon    -1.244   0.314  1.872          (------------*-----------)
                             --------+---------+---------+---------+-
                                  -1.2       0.0       1.2       2.4


Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Ecotype

Simultaneous confidence level = 88.31%


Ecotype = Arabidopsis subtracted from:

Ecotype    Lower  Center  Upper  ---------+---------+---------+---------+
Shandong  -1.900  -0.621  0.659  (------------*-----------)
Yukon     -1.586  -0.307  0.973     (------------*-----------)
                                 ---------+---------+---------+---------+
                                       -1.0       0.0       1.0       2.0


Ecotype = Shandong subtracted from:

Ecotype   Lower  Center  Upper  ---------+---------+---------+---------+
Yukon    -0.965   0.314  1.593           (------------*-----------)
                             ---------+---------+---------+---------+
                                   -1.0       0.0       1.0       2.0
```

Yukon Regime Gas phase respiration,

Descriptive Statistics: Q10

Results for Acclimation = Cold-acclimated

Variable	Ecotype	Mean	SE Mean
Q10	*Arabidopsis*	1.622	0.104
	Shandong	1.4596	0.0608
	Yukon	1.821	0.133

Results for Acclimation = Non-acclimated

Variable	Ecotype	Mean	SE Mean
Q10	*Arabidopsis*	1.697	0.224
	Shandong	1.3672	0.0733
	Yukon	1.3751	0.0926

Two-way ANOVA: Q10 versus Acclimation, Ecotype

Source	DF	SS	MS	F	P
Acclimation	1	0.21457	0.214569	2.23	0.146
Ecotype	2	0.39455	0.197275	2.05	0.147
Interaction	2	0.42480	0.212402	2.21	0.128
Error	30	2.88917	0.096306		
Total	35	3.92309			

S = 0.3103 R-Sq = 26.35% R-Sq(adj) = 14.08%